



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Chatterjee, D.K.

Appl. No. 09/558,421

Filed: April 26, 2000

For: **Mutant DNA Polymerases and  
Uses Thereof**

Art Unit: 1652

Examiner: Rao, M.

Atty. Docket: 0942.3600003/RWE/BJD

**Declaration of Deb K. Chatterjee  
Under 37 C.F.R. § 1.131(a)**

Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Deb K. Chatterjee, do hereby declare and say:

1. THAT, I, Deb Kumar Chatterjee, am the sole inventor of the subject matter claimed in the above-captioned patent application. I have read and understood the content of the above-captioned application, including the claims, which relates to mutant DNA polymerases and uses thereof. This application is, on information and belief, a continuation of Application No. 08/576,759, filed December 21, 1995 (parent application), which is a continuation of Application No. 08/537,397, filed October 2, 1995 (grandparent application), which is a continuation-in-part of Application No. 08/525,057, filed September 8, 1995 (great-grandparent application). I have also read and understood the content of the parent, grandparent and great-grandparent applications.

2. THAT, I hold the degree of Doctor of Philosophy. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

3. THAT, since 1986, I have been employed by Life Technologies, Inc. (LTI) (and now Invitrogen Corporation)<sup>1</sup>, the assignee of the above-captioned application, in the capacity of senior scientist from 1986-1988, group leader from 1988-1991, principal scientist from 1991-1992, research fellow from 1992-2000, and senior research fellow from 2000-present. See Exhibit A.

***I. Conception***

4. THAT, prior to October 17, 1994, I conceived of the invention claimed in above-captioned application. This conception related to mutant DNA polymerases comprising a single amino acid substitution in the O-helix. I conceived that the single amino acid substitution in the O-helix of the mutant DNA polymerases would be a Phe to Tyr substitution at a position corresponding to Phe<sup>667</sup> of wild-type *Taq* DNA polymerase.

In order to establish the conception of my invention before October 17, 1994, I submit herewith the following evidence.

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<sup>1</sup>Life Technologies, Inc. merged with Invitrogen Corporation on September 12, 2000, with Invitrogen Corporation being the surviving entity.

Attached hereto as Exhibit B is a copy of a paper by Lawyer *et al.*, *J. Biol. Chem.* 264:6427-6437 (1989), which describes the complete amino acid and coding sequences of the heat stable *Thermus aquaticus* ("*Taq*") DNA polymerase. I had this paper in my possession and was aware of these sequences before October 17, 1994.

A true copy of a page from my notebook is attached hereto as Exhibit C. This notebook page is dated prior to October 17, 1994, and was recorded prior to October 17, 1994, based on experiments conducted before October 17, 1994. The title of the experiment recorded on this notebook page is "*Taq* DNA Pol mutation." The purpose of this experiment was to create *Taq* DNA polymerase mutants having polymerase activity that utilize deoxy- or dideoxynucleotides more effectively. This notebook entry identifies a Tyr in T7 as an important amino acid residue, and indicates that this residue corresponds to Phe<sub>762</sub> in Klenow fragment DNA polymerase, and a Phe in *Taq* and T5 polymerase. Phe<sub>762</sub> of the Klenow fragment polymerase corresponds to Phe<sub>667</sub> of *Taq* polymerase and Phe<sub>570</sub> of T5 polymerase. (Specification at 8.)

A purpose of this notebook entry was to identify amino acid residues in the Klenow fragment, *Taq* polymerase, and T5 polymerase to be converted to the analogous amino acid in T7 polymerase (Tyr) in order to produce mutants that, among other things, exhibit less discrimination against dideoxynucleotides. Therefore, this notebook page describes a plan to construct mutant DNA polymerases comprising a Phe → Tyr substitution at a position in the O-helix corresponding to position 762 of *E. coli* polymerase I (Klenow fragment). This plan specifically includes constructing corresponding mutations in *Taq* polymerase and T5

polymerase, as well as *E. coli* polymerase I. In Exhibit C, an article citation has been masked, as well as dates.

A true copy of another page from my notebook is attached hereto as Exhibit D. This notebook page is dated prior to October 17, 1994, was recorded prior to October 17, 1994, based on experiments conducted before October 17, 1994. This notebook page describes a 33 nucleotide oligonucleotide sequence that was used to produce mutant "F667Y," a mutant *Taq* DNA polymerase with polymerase activity and non-discrimination properties. Mutant "F667Y" is a single amino acid substitution in *Taq* DNA polymerase converting Phe<sub>667</sub> to Tyr<sub>667</sub>. This position corresponds to position 762 of *E. coli* polymerase I.

This oligonucleotide was identified as "Sequence 2680," and contains a created *AseI* restriction site in addition to the nucleotide substitution, which results in mutant F667Y. These features can be observed directly on the sequence. The ATTAAT *AseI* restriction site is noted on the sequence. In addition, the sequence GTA, which is the complement of the Tyr codon TAC, can be observed immediately adjacent to the *AseI* restriction site. Since the full-length nucleotide and amino acid sequence of the wild-type *Taq* DNA polymerase gene and protein were known, *see supra*, the identification of this single amino acid substitution and the sequence of the synthetic oligonucleotide encoding this substitution also provides the full-length sequences for the F667Y mutant.

I ordered Sequence 2680 from Ms. Flora Lichaa prior to October 17, 1994, and I received the synthesized oligonucleotide from Ms. Lichaa prior to October 17, 1994. Therefore, prior to

October 17, 1994, I had a definite and permanent idea of a mutant *Taq* DNA polymerase with polymerase activity and non-discrimination properties comprising a phenylalanine to tyrosine substitution at position 667, including its full-length sequence.

## ***II. Actual Reduction to Practice***

THAT, after conceiving of the mutant *Taq* comprising a phenylalanine to tyrosine substitution at position 667, I supervised an effort to reduce this invention to practice by cloning and expressing a nucleic acid sequence encoding the mutant *Taq* polymerase. As a result of this effort, the mutant *Taq* polymerase was cloned and expressed prior to October 17, 1994. The experiments that led to the cloning and expression of the mutant *Taq* polymerase are described in more detail below.

Following receipt of the Sequence 2680, the oligonucleotide was kinased in a buffer containing 100 mM Tris, pH 8.0, 10 mM MgCl<sub>2</sub>, 5 mM DTT, 0.43 mM ATP, and 5 units of T4 kinase for 30 minutes at 37°C. A true copy of the laboratory notebook page describing this experiment is attached as Exhibit E. The notebook page is dated and signed by me and this experiment was completed prior to October 17, 1994.

After Sequence 2680 was kinased, it was used to mutagenize the *Taq* DNA polymerase gene. Sequence 2680 was annealed to a single-stranded DNA comprising the wild-type *Taq* DNA polymerase sequence at 70°C for 2 minutes followed by cooling to about 35°C over 30-40 minutes. The remaining portion of the DNA polymerase gene was synthesized with Sequence

2680 acting as a primer by incubation with T7 DNA polymerase and T4 ligase for 5 minutes on ice, 5 minutes at room temperature, followed by 45 minutes at 37°C. The resulting double-stranded DNA, one strand of which should encode the mutated *Taq* DNA polymerase gene, was used to transform DH5 $\alpha$ F'IQ bacteria. A true copy of the laboratory notebook page describing this experiment is attached as Exhibit F. This experiment was completed prior to October 17, 1994.

DNA was then isolated from clones of the transformed bacteria and assayed for the presence of an additional *AseI* restriction site, which was introduced into Sequence 2680. The presence of this *AseI* restriction site would indicate that the transformed bacterial clone contains DNA encoding the mutated *Taq* DNA polymerase sequence derived from Sequence 2680. A true copy of the laboratory notebook page describing an original screening for the *AseI* restriction site is attached as Exhibit G. A true copy of a laboratory notebook page describing an additional screening experiment is attached as Exhibit H. In this screening, DNA isolated from one clone was found to contain the additional *AseI* restriction site based on restriction fragment size following *AseI* digestion. This clone (#8) was saved. This notebook entry was dated and signed by me and this experiment was completed prior to October 17, 1994.

The DNA of clone 8 was then isolated, restricted with *NgoAIV* and *XbaI*, and inserted into plasmid pTQ18-*Taq* (to replace the wild-type fragment), an expression vector containing the *tac* promoter, which also had been digested with *NgoAIV* and *XbaI*. The resulting recombinant plasmid containing the mutant polymerase gene operably linked to the *tac* promoter was used to transform bacteria. The resulting recombinant plasmids, isolated from several transformed

bacterial clones, were assayed for the presence of the additional *AseI* restriction site to indicate incorporation of sequences derived from the synthetic oligonucleotide. Clones 1-3 and 6-10 were determined to contain the additional *AseI* restriction site, showing incorporation of the Sequence 2680 oligonucleotide sequence into the *Taq* DNA polymerase gene present in this plasmid. A true copy of the laboratory notebook pages describing this experiment is attached as Exhibit I. The notebook entry was dated and signed by me and this experiment was completed prior to October 17, 1994.

Bacterial cultures transformed with this plasmid, encoding the mutant *Taq* DNA polymerase gene as determined by the presence of an extra *AseI* restriction site, were cultured in the presence of IPTG. IPTG induces expression from the *tac* promoter, which is operably linked to the mutant *Taq* DNA polymerase gene having a Phe to Tyr mutation at position 667, in the recombinant expression vector. Thus, IPTG would induce expression of the mutant *Taq* polymerase. The resulting bacterial cultures following induction were given to Adam Goldstein to assay for heat stable DNA polymerase activity. See Exhibit I, page 2. Since *Taq* polymerase is a heat stable DNA polymerase, this single amino acid substituted mutant would also have heat stable polymerase activity. Therefore, the presence of heat stable DNA polymerase activity in the cultures is indicative of the expression of the mutant *Taq* polymerase.

This mutant was expressed and found to have polymerase activity prior to October 17, 1994. Enclosed as Exhibit J is a copy of Adam Goldstein's notebook entry regarding an experiment testing samples from these bacterial cultures, in which the mutant F667Y gene was induced, for polymerase activity. On information and belief, this notebook page was recorded

prior to October 17, 1994, and the experiment was conducted prior to October 17, 1994. I have reviewed the experimental data recorded in this notebook entry. These results indicate that samples from the transformed and induced bacterial samples contain heat stable DNA polymerase activity. Since the mutant *Taq* DNA polymerase would be the only heat stable DNA polymerase in these samples, this result indicates to me that *Taq* mutant F667Y was expressed and shown to have polymerase activity prior to October 17, 1994.

At the time the experiment described in Exhibit J was performed, I supervised Adam Goldstein's work relating to the cloning and expression of thermostable DNA polymerase, and had contact or discussions with Adam Goldstein nearly every day regarding this project. Therefore, I believe the results of this experiment were communicated to me soon after they were obtained and before October 17, 1994. Upon reviewing these results, I appreciated that the mutant *Taq* polymerase had been expressed and shown to have polymerase activity.

### ***III. Diligence***

THAT, following my conception of the claimed invention, I supervised a diligent effort to reduce this invention to practice. This diligent effort began before October 17, 1994, and included the cloning and expression of the mutant *Taq* DNA polymerase described *supra*. While the *Taq* DNA polymerase had been cloned and expressed prior to October 17, 1994, diligent activity involving *Tne*, *Tma*, and *Taq* polymerases continued after October 17, 1994, until the filing of the great-grandparent application on September 8, 1995.



This diligent effort included several different activities. As one step, the properties of several different wild-type DNA polymerases were characterized including T5, *Thermotoga neapolitana* (Tne), *Thermotoga maritima* (Tma), *Thermus flavus* (Tfl), *Thermus thermophilus* (Tth) and the Klenow fragment of *Escherichia coli* DNA polymerase I. A second step involved the cloning and expression of the wild-type genes encoding these polymerases. A third step involved introducing mutations into the genes encoding the polymerases. A fourth step involved transforming host cells with the mutant polymerase genes, expressing the mutant polymerases, and characterizing the biological activity of the mutant polymerases.

Mutations introduced in the DNA polymerases included mutations in the 5'-3' exonuclease domain to reduce or eliminate 5'-3' exonuclease activity, mutations in the 3'-5' exonuclease domain to reduce or eliminate 3'-5' exonuclease activity, in addition to mutations in the O-helix. More specifically, the mutant polymerase includes a mutation in a tyrosine residue at an amino acid position corresponding to position 667 of the *Taq polymerase* of the wild-type DNA polymerase. This position is located in the O-helix of Pol I-type DNA polymerases. The specific position of the wild-type DNA polymerase is, for example: *E. coli* bacteriophage T5 (T5) DNA polymerase I residue 570, *E. coli* (Klenow fragment) DNA polymerase I residue 762, *E. coli* bacteriophage T7 (T7) DNA polymerase I residue 526, *Thermotoga neapolitana* (Tne) DNA polymerase residue 67 of the sequence depicted in Figure 4 of the specification, *Thermotoga maritima* (Tma) DNA polymerase residue 730, *Streptococcus pneumoniae* DNA polymerase residue 711, *Thermus flavus* (Tfl) DNA polymerase residue 666, *Thermus thermophilus* DNA polymerase residue 669, *Deinococcus radiodurans* DNA polymerase residue 747, *Bacillus caldotenax* DNA polymerase residue 711, mycobacteriophage

L5 DNA polymerase residue 438, *E. coli* bacteriophage SP01 DNA polymerase 692 and *E. coli* bacteriophage SP02 DNA polymerase 447. As described in the specification, the polymerase can be modified to reduce 3' to 5' exonuclease activity and 5' to 3' exonuclease activity in addition to the mutation in the O-helix.

During the period from just prior to October 17, 1994, until September 8, 1995, individuals under my supervision worked on the cloning, expression, and characterization of wild-type and mutant DNA polymerases. These individuals include Carolyn Combs, Barbara Flynn, Elizabeth Flynn, Adam Goldstein, A. John Hughes, Jr., Roger Lasken, Flora Lichaa, Mary Longo, Avani Patel, Brian Schmidt, Harini Shandilya and Kalavathy Sitaraman. As part of my responsibilities at Life Technologies, I would discuss experiments to be performed and would be kept informed of the results of these experiments on a regular basis. For example, from October 1994 until September 1995 it was my practice to conduct weekly laboratory meetings. All of the individuals listed above typically attended these weekly laboratory meeting, which were typically held on Monday mornings. During this time period, recently obtained results relating to the DNA polymerase project were often discussed.

I have reviewed the laboratory notebooks of the individuals working on the project. Based on these laboratory notebook records and my recollection, the following activity relating to the DNA polymerase project took place under my supervision from October 16, 1994, until September 8, 1995.

On or about October 13, 1994, Brian Schmidt performed an experiment digesting a mutant *Tne* DNA with various restriction enzymes. This experiment was recorded on pages 49-50 of notebook 3884. A copy of thereof is attached as Exhibit S-1.

On or about October 18, 1994, Roger Lasken performed an experiment relating to the optimal conditions for the use of thermostable polymerases. In this experiment varying amounts of *Taq* polymerase were added to a DNA template to saturate the 3' end in a polymerase assay. The experiment suggested that *Taq* polymerase binding to DNA exhibits an equilibrium effect, and provided information regarding sequencing conditions with wild-type *Taq* polymerase. This experiment was recorded on pages 65-66 of notebook 3902. A copy thereof is attached as Exhibit L-1.

On or about October 19, 1994, Roger Lasken performed an experiment investigating the optimal conditions for the use of thermostable polymerases in long PCR. In this experiment he labeled primers for long PCR with <sup>32</sup>P to optimize the *Tfl* polymerase concentration in long PCR reactions using mixtures of *Tfl* and Vent® polymerases. This experiment was used to provide additional information regarding sequencing conditions with *Tfl* polymerase. This experiment was recorded on page 67 of notebook 3902. A copy thereof is attached as Exhibit L-2 .

On or about October 24, 1994, Roger Lasken began an experiment investigating the use of *Tfl* polymerase in long PCR reactions. A purpose of this experiment was to optimize the concentrations of *Tfl* polymerase in a mixture of *Tfl* and Vent® polymerases, for use in long PCR reactions, and to provide additional information regarding sequencing conditions with *Tfl*

polymerase. This experiment was recorded on page 67 of notebook 3902. A copy thereof is attached as Exhibit L-3.

On or about October 24, 1994, Roger Lasken continued his experiment on the use of *Tfl* polymerase in long PCR reactions. In this experiment he ran the samples from the reactions performed on October 24, 1994 on an ethidium bromide stained 8% agarose gel. This experiment was recorded on pages 67-69 of notebook 3902. A copy thereof is attached as Exhibit L-4.

On or about October 25, 1994, Roger Lasken performed an experiment comparing the apparent molecular weights of a number of thermostable polymerases. In this experiment he ran an SDS-PAGE gel of eight thermostable DNA polymerases: *Tfl* (epicenter), *Tfl* (MBR), *Tth* (MBR), *rTth* (Perkin Elmer), sequitherem (epicenter), Vent® (NEB), DeepVent® (NEB), and *Taq* EKBT1. This experiment was recorded on pages 72-73 of notebook 3902. A copy thereof is attached as Exhibit L-5 .

On or about October 26, 1994, Roger Lasken continued his experiments on the use of *Tfl* polymerase in long PCR reactions. In this experiment he ran samples from reactions run on October 24, 1994, on an SDS page gel to determine the amount of degradation of the radioactive product. The gel was exposed to a phosphoimager plate overnight. This experiment was recorded on pages 70-71 of notebook 3902. A copy thereof is attached as Exhibit L-6.

On or about October 27, 1994, Roger Lasken continued his experiments on the use of *Tfl* polymerase in long PCR reactions. In this experiment he analyzed the data from the autoradiograph exposed overnight, and noted only slight degradation of product after 36 cycles. This experiment was recorded on pages 70-71 of notebook 3902. A copy thereof is attached as Exhibit L-7.

On or about October 28, 1994, Roger Lasken made a 2.5 unit/ $\mu$ l stock of recombinant *Taq* (of EKBT1) by 1:1 dilution of a 5 unit/ $\mu$ l stock. This activity was recorded on page 74 of notebook 3902. A copy thereof is attached as Exhibit L-8.

On or about November 1, 1994, Roger Lasken prepared additional experiments on the use of *Tfl* polymerase in long PCR reactions. He prepared labeled primers for a *Tfl*/Vent® primer degradation assay. Primers were prepared with  $\alpha$ S and ribo ends. This activity was recorded on page 75 of notebook 3902. A copy thereof is attached as Exhibit L-9.

On or about November 2, 1994, Roger Lasken continued his experiments on the use of *Tfl* polymerase in long PCR reactions. In this experiment he performed primer degradation assays using mixtures of *Tfl* and Vent® polymerases. This experiment was recorded on page 76 of notebook 3902. A copy thereof is attached as Exhibit L-10.

On or about November 2, 1994, Roger Lasken performed an experiment investigating the optimal conditions for use of *Tne* polymerase in DNA sequencing. He set up sequencing reactions with *Tne* polymerase in Vent® and Cheng buffers. The reactions were analyzed by SDS

PAGE, and exposed to a phosphoimager plate. The experiment showed that reactions did not sequence information when performed in Cheng buffer. This experiment was recorded on pages 77-78 of notebook 3902. A copy thereof is attached as Exhibit L-11.

On or about November 4, 1994, Roger Lasken began experiments comparing the 3' to 5' exonuclease activity of *Tne* polymerase. He performed primer degradation assays on *Tne*, Vent®, and DeepVent® polymerases as controls in Cheng, Vent® and Klentaq buffers. He exposed phosphoimager plate overnight. This experiment was recorded on pages 80-81 of notebook 3902. A copy thereof is attached as Exhibit L-12.

On or about November 5, 1994, Roger Lasken developed the autoradiograph image from the plate exposed on November 4, 1994, analyzed the data and prepared graphs depicting the data. The experiment showed that the turnover of *Tne* polymerase was approximately 2 times lower than both DeepVent® and Vent® polymerase. This experiment was recorded on pages 82-83 of notebook 3902. A copy thereof is attached as Exhibit L-13.

On or about November 7-9, 1994, Roger Lasken performed an experiment to investigate the effect of buffer conditions on the 5' to 3' exonuclease activities of a number of polymerases. In this experiment he performed turnover experiments using Vent®, DeepVent®, and *Tne* polymerases in Cheng, Klentaq and Vent® buffer. The experiment showed that the buffer effect on turnover was minimal. This experiment was recorded on pages 84-85 of notebook 3902. A copy thereof is attached as Exhibit L-14.

On or about November 10, 1994, Roger Lasken further analyzed data from the experiment performed on November 9, 1994. This activity was recorded on pages 86-87 of notebook 3902. A copy thereof is attached as Exhibit L-15.

On or about November 15, 1994, Roger Lasken performed a quality control experiment on recombinant *Taq* polymerase (*rTaq*) stocks for use as controls in additional experiments on the use of thermostable polymerases in sequencing and PCR. In this quality control experiment he determined unit activity concentration. This experiment was recorded on pages 90-91 of notebook 3902. A copy thereof is attached as Exhibit L-16.

On or about November 16, 1994, Kalavathy Sitaraman performed an experiment testing the ability of certain primers to amplify GAPDH (glyceraldehyde-3-phosphate dehydrogenase). In this experiment, DeepVent® polymerase (plus and minus exonuclease activity), and various primers, were added to a DNA template. This activity was recorded on pages 96-97 of notebook 3831. A copy thereof is attached as Exhibit K-1.

On or about November 18, 1994, Kalavathy Sitaraman performed an experiment to determine the optimal concentrations of various polymerases to be used in amplifying DNA templates. In this experiment, various concentrations of *Taq*, DeepVent® and *Taq* + DeepVent® polymerases were added to a pMC9 DNA template. This activity was recorded on pages 99-100 of notebook 3831. A copy thereof is attached as Exhibit K-2.

On or about November 21, 1994, Kalavathy Sitaraman continued experiments to amplify pMC9. In this experiment, deoxyuracil-containing primers and a combination of *Taq* and DeepVent® polymerases were added to a pMC9 DNA template. She also tested Klenow *Taq* (KT) and DeepVent® (DV) buffers by looking at the amount of product yielded by the reaction, as well as the fidelity of the primers. This experiment suggested that KT is a better buffer than DV and that a 1:0.01 unit ratio of *Taq* to DeepVent® is better than *Taq* alone. This activity was recorded on pages 101-102 of notebook 3831. A copy thereof is attached as Exhibit K-3.

On or about November 21, 1994, Kalavathy Sitaraman continued experiments to amplify pMC9. In this experiment, 1 unit of *Taq* and varying concentrations of DeepVent® polymerases were added to a pMC9 DNA template to determine optimal conditions for reducing or preventing mispriming. This activity was recorded on pages 103-104 of notebook 3831. A copy thereof is attached as Exhibit K-4.

On or about November 21, 1994, Kalavathy Sitaraman continued experiments to amplify pMC9. In this experiment, deoxyuracil-containing primers and varying concentrations of DeepVent® polymerase were added to a pMC9 DNA template. These results indicated that DeepVent® alone did not amplify the template under the tested conditions. This activity was recorded on page 105 of notebook 3831. A copy thereof is attached as Exhibit K-5.

On or about November 22, 1994, Kalavathy Sitaraman continued experiments to amplify pMC9. In this experiment, primers lacking deoxyuracil (dU) and *Taq*, DeepVent®, or *Taq* + DeepVent® polymerases were added to a pMC9 DNA template. These results confirmed that



DeepVent® alone did not amplify template; the mispriming was virtually eliminated using *Taq* + DeepVent® polymerase with primers lacking dU; and that use of dU-containing primers resulted in the production of higher amounts of dimerized primers than did use of non-dU-containing primers. This activity was recorded on page 106-108 of notebook 3831. A copy thereof is attached as Exhibit K-6.

On or about November 28, 1994, Kalavathy Sitaraman experimented with various polymerase chain reaction (PCR) conditions to prevent mispriming during pMC9 amplification. In this experiment, three different sets of primers were tested, and annealing temperatures and polymerase (*Taq*, DeepVent®, or *Taq* + DeepVent®) amounts were varied. This activity was recorded on page 109-110 of notebook 3831. A copy thereof is attached as Exhibit K-7.

On or about November 29, 1994, Kalavathy Sitaraman prepared and tested a new type of agarose for preparing gels. Samples tested were reaction products from the experiments described at pages 109-110 of notebook 3831 (*see* Exhibit K-7). This activity was recorded on page 111 of notebook 3831. A copy thereof is attached as Exhibit K-8.

On or about November 30, 1994, Kalavathy Sitaraman continued experiments in which PCR conditions were varied, to determine optimal conditions for prevention of mispriming during pMC9 amplification. In this experiment, template concentrations, Mg<sup>++</sup> concentrations, dNTP concentrations, and number of amplification cycles were varied during pMC9 amplification using *Taq*, DeepVent® or *Taq* + DeepVent® polymerases. This activity was recorded on pages 112-113 of notebook 3831. A copy thereof is attached as Exhibit K-9.

On or about November 30, 1994, Kalavathy Sitaraman conducted experiments in which pGAPDH was amplified using *Taq*, DeepVent® and *Taq* + DeepVent® polymerases. The results suggested that DeepVent® alone at low concentrations gave the best product yield. This activity was recorded on pages 114-115 of notebook 3831. A copy thereof is attached as Exhibit K-10.

On or about November 30, 1994, Roger Lasken prepared EKBT1 r*Taq* dilutions (10 u/ $\mu$ l) for use by other employees of LTI in product scale-up development. This activity was recorded on pages 82-87 and 90-92 of notebook 3902. A copy thereof is attached as Exhibit L-17.

On or about December 1, 1994, Kalavathy Sitaraman continued experiments in which pMC9 was amplified. In this experiment, new deoxyuracil-containing primers were tested and Mg<sup>++</sup> concentrations were varied to optimize reaction conditions that would prevent the mispriming of the pMC9 template. This activity was recorded on pages 116-117 of notebook 3831. A copy thereof is attached as Exhibit K-11.

On or about December 1, 1994, Kalavathy Sitaraman continued experiments to optimize the conditions for amplifying pMC9. In this experiment, the concentration of *Taq* polymerase was increased from 1 unit to 2 units. This activity was recorded on page 118 of notebook 3831. A copy thereof is attached as Exhibit K-12.

On or about December 4, 1994, Kalavathy Sitaraman performed experiments to determine optimal reaction conditions for UDG cloning of PCR reaction products. Concentrations of UDG and PCR products were titrated and the reaction mixtures were used to

transform MaxEfficiency® DH5α *E. coli* host cells. This activity was recorded on pages 120-121 of notebook 3831. A copy thereof is attached as Exhibit K-13.

On or about December 5-7, 1994, Adam Goldstein conducted experiments to prepare a purified preparation of the F667Y mutant of *Taq* polymerase. In this experiment, Adam Goldstein lysed cells containing the mutant protein, performed PEI and ammonium sulfate precipitations on the lysate, and dialyzed the pellet. The polymerase was further purified on a heparin column. This experiment was recorded on page 100 of notebook 3865. A copy thereof is attached as Exhibit G-1.

On or about December 6, 1994, Kalavathy Sitaraman continued experiments to optimize the conditions for amplification of pMC9. In this experiment, 2 units of *Taq* or *Taq* + DeepVent® polymerases and non-deoxyuracil forward and reverse primers, were used to amplify pMC9. This activity was recorded on pages 122-123 of notebook 3831. A copy thereof is attached as Exhibit K-14.

On or about December 6, 1994, Kalavathy Sitaraman prepared the pUC19 vector. In this experiment, the vector was digested with the AatII, EcoRI, BamHI, and AflIII restriction enzymes. A diagnostic gel was run to ensure the proper restriction pattern. Unrestricted pUC19 was gel-purified and ethanol-precipitated. This activity was recorded on pages 124-125 of notebook 3831. A copy thereof is attached as Exhibit K-15.

On or about December 7, 1994, Kalavathy Sitaraman performed experiments to transform bacterial cells with pUC19 vector amplified using forward and reverse non-deoxyuracil primers, and varying amounts of either *Taq* or *Taq* + DeepVent® polymerases. This activity was recorded on page 126 of notebook 3831. A copy thereof is attached as Exhibit K-16.

On or about December 8, 1994, Kalavathy Sitaraman performed experiments to amplify pUC19 vector that had been linearize by digestion with XmnI. Amplification was performed with either *Taq* or *Taq* + DeepVent® polymerases, and the concentration of  $Mg^{++}$  was titrated in the reaction mixtures. The PCR products from duplicate samples were pooled, phenol-extracted and ethanol precipitated. The amount of DNA was then quantified on a gel, DNA was then cut with a restriction enzyme, and an 875 bp insert was gel-purified. This activity was recorded on pages 127-130 of notebook 3831. A copy thereof is attached as Exhibit K-17.

On or about December 8, 1994, Roger Lasken began an experiment to study conditions for the use of *Taq* and Vent® polymerases in long PCR reactions. In this experiment he performed PCR reactions using mixtures of *Taq* and DeepVent® polymerases with no  $Mn^{2+}$  or dNTP bias. He received plates of cells that contained the pUC19 vector which would serve as the template for the PCR assays and picked 20 white colonies and 2 blue colonies. He inoculated them into LB media with ampicillin, and grew them overnight. This experiment was recorded on page 93 of notebook 3902. A copy thereof is attached as Exhibit L-18.

On or about December 9, 1994, Roger Lasken continued the experiment on conditions for *Taq* polymerase long PCR reactions by preparing the template for the long PCR experiments.

He performed minipreps on the cultures grown overnight on December 8, 1994, and analyzed them by restriction mapping. This experiment was recorded on page 93 of notebook 3902. A copy thereof is attached as Exhibit L-19.

On or about December 10, 1994, Adam Goldstein continued his experiment to prepare a purified preparation of the F667Y mutant of *Taq* polymerase. He analyzed data from the heparin column and assayed fractions from this column for polymerase activity. The fractions containing activity were pooled and dialyzed. This experiment was recorded on page 101 of notebook 3865. A copy thereof is attached as Exhibit G-2.

On or about December 12, 1994, Kalavathy Sitaraman performed experiments to ligate the purified vector and insert from the experiment conducted on December 8, 1994 (*see* Exhibit K-17). Vector and insert were amplified in the presence of *Taq* or *Taq* + DeepVent® polymerases, or in the absence of polymerase (controls). *E. coli* DH5α MaxEfficiency® cells were transformed with the ligated products. This activity was recorded on page 131 of notebook 3831. A copy thereof is attached as Exhibit K-18.

On or about December 12, 1994, Elizabeth Flynn continued the experiment to prepare a purified preparation of the F667Y mutant of *Taq* polymerase. She washed and equilibrated a Super Q 650 column, and added the dialysate from the experiment performed on December 10, 1994, to further purify the F667Y *Taq* polymerase mutant. The fractions from the Q650 column were assayed for polymerase activity, and the active fractions were pooled. This experiment was recorded on pages 102-103 of notebook 3865. A copy thereof is attached as Exhibit F-1.

Soon after December 10, 1994, Roger Lasken began an experiment to determine the heat stable polymerase activity of recombinantly expressed *Tfl*. He obtained seven clones containing the *Tfl* polymerase gene from Ayoub Rashtchian, another LTI employee, inoculated the cells into LB and induced expression. Cultures were centrifuged and the cell pellets were stored at -70°C overnight. This experiment was recorded on page 94 of notebook 3902. A copy thereof is attached as Exhibit L-20.

On or about December 13, 1994, Kalavathy Sitaraman ran the vector, insert and ligation reaction products (*see* Exhibit K-18) on a gel to determine if the ligation was successful. Bacteria were re-transformed with the products of the *Taq* and *Taq* + DeepVent® reactions and replated. Mini-preps were prepared from colonies picked from plates that had received *Taq* or *Taq* + DeepVent® samples. This activity was recorded on pages 132-133 of notebook 3831. A copy thereof is attached as Exhibit K-19.

On or about December 13, 1994, Kalavathy Sitaraman continued experiments to optimize conditions for ligation of vector and insert. The ligation reaction was performed with a different ratio of vector to insert from that in the previous experiments, and *E. coli* DH5α cells were transformed with the product. This activity was recorded on page 134 of notebook 3831. A copy thereof is attached as Exhibit K-20.

On or about December 13-14, 1994, Elizabeth Flynn continued the experiment to prepare a purified preparation of the *Taq* F667Y mutant of *Taq* polymerase. She optimized the time

course and dilutions for the mutant F667Y *Taq*. This experiment was recorded on pages 104-106 of notebook 3865. A copy thereof is attached as Exhibit F-2.

On or about December 14, 1994, Kalavathy Sitaraman replated the transformed cells left over from the December 13, 1994, transformation reaction (*see* Exhibit K-20). This activity was recorded on page 135 of notebook 3831. A copy thereof is attached as Exhibit K-21.

On or about December 14, 1994, Kalavathy Sitaraman used imbalance reaction mixtures from Ayoub Rashtchian, another employee of LTI, in the transformation step, to determine if better transformant/mutant colony numbers could be obtained. This activity was recorded on pages 136-137 of notebook 3831. A copy thereof is attached as Exhibit K-22.

On or about December 14, 1994, Roger Lasken continued his experiment to determine the heat stable polymerase activity of recombinantly expressed *Tfl* polymerase. He lysed and heat-treated host cell clones containing a recombinant *Tfl* gene, and assayed the lysates for polymerase activity. All clones were shown to contain heat stable polymerase activity. This experiment was recorded on page 95 of notebook 3902. A copy thereof is attached as Exhibit L-21.

On or about December 15, 1994, Roger Lasken continued his experiment to determine the heat stable polymerase activity of recombinantly expressed *Tfl* polymerase. He analyzed the results from the heat stable polymerase assay performed on December 14, 1994, and prepared graphs. Additionally, he labeled primers for a further experiment to determine whether different

polymerases can incorporate thymidine into a new DNA strand using a uracil containing template. These experiments were recorded on pages 97-99 of notebook 3902. A copy thereof is attached as Exhibit L-22.

On or about December 16, 1994, Kalavathy Sitaraman counted blue and white colonies to determine transformation efficiency from the *Taq* and *Taq* + DeepVent® reactions. This activity was recorded on page 138 of notebook 3831. A copy thereof is attached as Exhibit K-23.

On or about December 16, 1994, Kalavathy Sitaraman performed experiments to determine if all of the white colonies obtained in the previous experiment (*see* Exhibit K-23) were full-length clones, by miniprepping overnight cultures prepared from white colonies picked from the previous experiment. This activity was recorded on page 139-140 of notebook 3831. A copy thereof is attached as Exhibit K-24.

On or about December 16, 1994, Roger Lasken continued his experiment to determine whether different polymerases can incorporate thymidine into a new DNA strand using a uracil containing template. He performed assays for thymidine incorporation using a uracil containing template with Ultima, *Taq*, *Tne*, *Tfl*, *Tth*, Vent®, DeepVent®, Pfu and DTOK polymerases. Gels were run and exposed to a phosphoimager plate overnight. This experiment was recorded on pages 100-101 of notebook 3902. A copy thereof is attached as Exhibit L-23.

On or about December 17, 1994, Roger Lasken continued his experiment to determine whether different polymerases can incorporate thymidine into a new DNA strand using a uracil



containing template by analyzing data from the thymidine incorporation assays conducted December 15, 1994, and December 16, 1994. The experiment was not completed. This experiment was recorded on pages 102-103 of notebook 3902. A copy thereof is attached as Exhibit L-24.

On or about December 19, 1994, Kalavathy Sitaraman continued experiments to amplify pMC9, to determine fidelity of amplification with varying amounts of *Taq* or *Taq* + DeepVent® polymerases. In this experiment, the concentration of template was varied, and template was amplified using 1 or 2 units of polymerase. This activity was recorded on pages 141-142 of notebook 3831. A copy thereof is attached as Exhibit K-25.

On or about December 20, 1994, Kalavathy Sitaraman continued experiments to optimize the conditions for amplifying pMC9, using two units of *Taq* polymerase and varying concentrations of DeepVent® polymerase. This activity was recorded on page 143 of notebook 3831. A copy thereof is attached as Exhibit K-26.

On or about December 27, 1994, Kalavathy Sitaraman reran the samples from the December 20-22, 1994, experiment (*see* Exhibit K-26) on an agarose gel. This activity was recorded on page 144 of notebook 3831. A copy thereof is attached as Exhibit K-27.

On or about December 27, 1994, Kalavathy Sitaraman repeated the experiment from December 20-22, 1994 (*see* Exhibit K-26), by attempting to amplify pMC9 using 2 units of *Taq*

polymerase and varying concentrations of DeepVent® polymerase. This activity was recorded on pages 145-146 of notebook 3831. A copy thereof is attached as Exhibit K-28.

On or about December 27, 1994, Kalavathy Sitaraman continued experiments to amplify pMC9 using varying ratios of *Taq* + DeepVent® polymerases or varying concentrations of *Taq* or DeepVent® polymerase alone. This activity was recorded on page 147 of notebook 3831. A copy thereof is attached as Exhibit K-29.

On or about December 27, 1994, Kalavathy Sitaraman continued experiments to amplify pMC9 using *Taq* + DeepVent® polymerases in a ratio of 2 units of *Taq* polymerase to 0.2 units of DeepVent® polymerase and varying concentrations of  $Mg^{++}$ . This activity was recorded on pages 148 of notebook 3831. A copy thereof is attached as Exhibit K-30.

On or about December 28, 1994, to January 3, 1995, Kalavathy Sitaraman continued experiments to amplify pMC9 using varying concentrations of *Taq* and DeepVent® polymerases. The effect of freshly preparing the enzyme mixture and adding it separately was also determined. This activity was recorded on pages 149-150 of notebook 3831. A copy thereof is attached as Exhibit K-31.

On or about January 3-4, 1995, Kalavathy Sitaraman pooled the products from the December 27 and December 28 reactions (*see* Exhibits K-28 through K-31), and extracted and purified the DNA. This activity was recorded on page 151 of notebook 3831. A copy thereof is attached as Exhibit K-32.

On or about January 3-5, 1995, Kalavathy Sitaraman started bacterial cultures to propagate different sized fragments of pMC9 in pDELTA1. This activity was recorded on page 152 of notebook 3831. A copy thereof is attached as Exhibit K-33.

On or about January 5, 1995, Kalavathy Sitaraman attempted to amplify the 6.4 kb and 8.0 kb pMC9 fragments using *Taq* and DeepVent® polymerases at a 1:0.01 concentration ratio. This activity was recorded on pages 153-154 of notebook 3831. A copy thereof is attached as Exhibit K-34.

On or about January 5, 1995, Kalavathy Sitaraman attempted to optimize conditions for the amplification of the 6.4 kb pMC9 fragment using *Taq* polymerase alone, or mixtures of *Taq* + DeepVent® polymerases at varying concentration ratios. This activity was recorded on pages 155-158 of notebook 3831. A copy thereof is attached as Exhibit K-35.

On or about January 9, 1995, Kalavathy Sitaraman attempted to amplify the 10.5 kb pMC9 fragment contained in pDELTA1, using varying amounts of *Taq* + DeepVent® polymerases and varying concentrations of  $Mg^{++}$ . This activity was recorded on pages 159-160 of notebook 3831. A copy thereof is attached as Exhibit K-36.

On or about January 9, 1995, Kalavathy Sitaraman grew bacterial host cells that had been transformed with amplified fragments of different sizes, under more stringent conditions, in order to decrease the number of bacterial colonies on each plate. This activity was recorded on page 161 of notebook 3831. A copy thereof is attached as Exhibit K-37.

On or about January 11, 1995, Roger Lasken performed an experiment to determine the molecular weight of *rTaq* and native *Taq* polymerases by SDS polyacrylamide gel electrophoresis (SDS-PAGE). This experiment was recorded on page 104 of notebook 3902. A copy thereof is attached as Exhibit L-25.

On or about January 12, 1995, Roger Lasken prepared more template to be used in PCR experiments using various DNA polymerase to investigate fidelity of amplification using *Taq* or mixtures of *Taq* + DeepVent®. pUC19 clones were prepared for use as template. Additionally, he began restriction mapping the plasmids to confirm the identity of the clones. This experiment was recorded on page 106 of notebook 3902. A copy thereof is attached as Exhibit L-26.

On or about January 13, 1995, Roger Lasken continued restriction mapping the pUC clones that he began on January 12, 1995. This activity was recorded on page 107 of notebook 3902. A copy thereof is attached as Exhibit L-27.

On or about January 16, 1995, Roger Lasken outlined ideas and conditions for assays to measure the ability of a polymerase to incorporate thymidine into a new DNA strand from a uracil containing template. Primers were radiolabeled for these assays. This experiment was recorded on pages 108-109 of notebook 3902. A copy thereof is attached as Exhibit L-28.

On or about January 17, 1995, Roger Lasken continued his experiment to measure the ability of a polymerase to incorporate thymidine into a new DNA strand from a uracil containing template. He performed the thymidine incorporation assays using recombinant *Taq* and Vent®

polymerases. This activity was recorded on page 110 of notebook 3902. A copy thereof is attached as Exhibit L-29.

On or about January 18, 1995, Roger Lasken continued his experiment to measure the ability of a polymerase to incorporate thymidine into a new DNA strand from a uracil containing template. He performed thymidine incorporation assays using SEQUENASE. This experiment was recorded on page 111 of notebook 3902. A copy thereof is attached as Exhibit L-30.

On or about January 19, 1995, Roger Lasken analyzed data from the thymidine incorporation assays performed on January 17-18, 1995. This analysis was recorded on page 112 of notebook 3902. A copy thereof is attached as Exhibit L-31.

On or about January 25, 1995, Brian Schmidt outlined ideas for recloning the *Tne* polymerase gene fragment into M13 for mutagenesis. Additionally, he restricted pSport-*Tne* to confirm the identity of this plasmid. This activity was recorded on pages 51-52 of notebook 3884. A copy thereof is attached as Exhibit S-2.

On or about January 26, 1995, Brian Schmidt began an experiment to clone the *Tne* polymerase gene fragment into M13. He redigested pSport-*Tne* to confirm the identity of the plasmid. Preparative digests of pSport-*Tne* and M13mp19 were also performed. The fragments were ligated and used to transform DH10B host cells by electroporation. This experiment was recorded on pages 53-55 of notebook 3884. A copy thereof is attached as Exhibit S-3.

On or about January 27, 1995, Roger Lasken began preparing template for various polymerase experiments, including the fidelity assays. He also prepared pUC clones to be used in PCR fidelity assays. This experiment was recorded on page 114 of notebook 3902. A copy thereof is attached as Exhibit L-32.

On or about January 30, 1995, Roger Lasken digested the pUC clones purified on January 27, 1995, with restriction enzymes to confirm their identity. This experiment was recorded on page 115 of notebook 3902. A copy thereof is attached as Exhibit L-33.

On or about January 31, 1995, Roger Lasken performed experiments to investigate the fidelity of recombinant *Taq* polymerase in long PCR reactions. In this experiment he performed PCR fidelity assays using recombinant *Taq* with and without Vent® polymerase in the presence of various concentrations of  $Mn^{++}$ . Reaction products were run on an agarose gel, and the results analyzed. New dilutions of recombinant *Taq* polymerase were prepared. This experiment was recorded on pages 116-119 of notebook 3902. A copy thereof is attached as Exhibit L-34.

On or about February 1, 1995, A. John Hughes, Jr., performed a quality control experiment on an aliquot of *Tne* polymerase. He determined the unit activity concentrations of two *Tne* polymerase samples to insure their quality. This activity was recorded on page 51 of notebook 3875. A copy thereof is attached as exhibit H-1.

On or about February 1, 1995, Roger Lasken performed a quality control experiment to aid in the investigation of the use of *Taq* polymerase. In this experiment he determined the

pipette accuracy in order to optimize *Taq* polymerase storage pipetting. This experiment was recorded on page 120 of notebook 3902. A copy thereof is attached as Exhibit L-35.

On or about February 2, 1995, Roger Lasken performed an experiment to confirm the identity of mutants from the fidelity experiment conducted on January 31, 1995, using restriction mapping. This experiment was recorded on pages 123-124 of notebook 3902. A copy thereof is attached as Exhibit L-36.

On or about February 3, 1995, Roger Lasken performed an experiment to investigate the stability of recombinant *Taq* polymerase in various storage conditions. In this experiment he prepared recombinant *Taq* solutions with various buffers, and measured the polymerase activities of these solutions to serve as a baseline for future stability studies. This experiment was recorded on pages 121-122 of notebook 3902. A copy thereof is attached as Exhibit L-37.

On or about February 7, 1995, Brian Schmidt began an experiment to clone a *Tne* gene fragment. Instead of using the *Bam*HI/*Sph*I digest he used an *Sph*I digest in this experiment and would select for directionality using restriction mapping. pSport-*Tne* and M13mp19 were digested with restriction enzymes. The resulting restriction fragments were ligated together and used to transform *E. coli*. This experiment was recorded on pages 56-57 of notebook 3884. A copy thereof is attached as exhibit S-4.

On or about February 8, 1995, Brian Schmidt continued the experiment to clone a *Tne* gene fragment into M13. He digested new constructs ligated on February 7, 1995, with

restriction endonucleases to select for the proper directionality of the insert. This experiment was recorded on pages 58-59 of notebook 3884. A copy thereof is attached as Exhibit S-5.

On or about February 8, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone the *Tne* gene into M13, from Brian Schmidt. Plasmid was purified from the *Tne* clones obtained on February 8, 1995. This experiment was recorded on page 1 of notebook 3966. A copy thereof is attached as Exhibit P-1.

On or about February 9, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* gene fragment into M13. M13 ssDNA was purified, and new media and plates were made. Additionally, new cultures of CJ236 and the new *Tne* clones were started. This experiment was recorded on pages 2-5 of notebook 3966. A copy thereof is attached as Exhibit P-2.

On or about February 10, 1995, Roger Lasken performed an experiment to optimize assay conditions for the primer degradation assays using Vent® polymerase. This experiment was recorded on pages 126-127 of notebook 3902. A copy thereof is attached as Exhibit L-38.

On or about February 13, 1995, Roger Lasken continued his investigations of the optimal conditions for the primer degradation assays by running a 16% SDS-PAGE gel on the samples from February 10, 1995. This experiment was recorded on page 128 of notebook 3902. A copy thereof is attached as Exhibit L-39.



On or about February 14, 1995, Roger Lasken continued his experiment to investigate the optimal conditions for the primer degradation assays by running additional gels and developing the phosphoimage from the experiments performed on February 10, 1995. He observed that increasing ionic strength in the buffer decreases the rate of degradation of the primers. This experiment was recorded on page 129 of notebook 3902. A copy thereof is attached as Exhibit L-40.

On or about February 15, 1995, Roger Lasken continued his experiment to investigate the optimal conditions for the primer degradation assays by determining the effects of potassium chloride concentration on the assays when using *Taq* and *Tne* polymerases. This experiment was recorded on pages 130-131 of notebook 3902. A copy thereof is attached as Exhibit L-41.

On or about February 16, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* polymerase gene fragment, by determining a restriction map for the new *Tne* constructs. This experiment was recorded on pages 6-8 of notebook 3966. A copy thereof is attached as Exhibit P-3.

On or about February 16, 1995, Brian Schmidt continued the experiment to clone a *Tne* polymerase gene fragment into M13 by assembling the restriction map of the T.nea/mp19 construct. This experiment was recorded on page 61 of notebook 3884. A copy thereof is attached as Exhibit S-6.

On or about February 16, 1995, Roger Lasken conducted an experiment to prepare a radioactive DNA ladder for use in later experiments. This experiment was recorded on page 132 of notebook 3902. A copy thereof is attached as Exhibit L-42.

On or about February 17, 1995, Roger Lasken continued his experiment to investigate the optimal conditions for the primer degradation assays using recombinant *Taq* and *Tne* polymerases by analyzing the data from the assays performed on February 15, 1995, and found that potassium chloride inhibits extension from the primed M13 template. This analysis was recorded on page 134 of notebook 3902. A copy thereof is attached as Exhibit L-43.

On or about February 20, 1995, Roger Lasken performed an experiment to prepare reagents for use in the primer degradation assays by radiolabeling primers to be used in the those assays. This experiment was recorded on page 136 of notebook 3902. A copy thereof is attached as Exhibit L-44.

On or about February 21, 1995, Roger Lasken performed an experiment to determine the extent of primer degradation under PCR conditions with different concentrations of KCl. This experiment was recorded on page 137 of notebook 3902. A copy thereof is attached as Exhibit L-45.

On or about February 21, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* gene fragment into M13. The *Tne* clones and M13 DNA were restricted for subsequent cloning. Potassium chloride was added and samples

were stored overnight. This experiment was recorded on pages 13-14 of notebook 3966. A copy thereof is attached as Exhibit P-4.

On or about February 21, 1995, Brian Schmidt requested the synthesis of a 26-mer mutant *Tne* oligonucleotide (designated oligo # 2899) and a 30-mer 5'-3' exonuclease mutant *Tne* oligonucleotide (designated oligo # 2900). Both oligos were synthesized by LTI personnel, with synthesis completed on February 24, 1995. This activity was recorded on page 23 of notebook 3964. A copy is attached as Exhibit S-7.

On or about February 22-23, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* gene fragment into M13. The reactions from February 21, 1995 (*see* Exhibit P-4), were analyzed by gel electrophoresis. Restriction fragments were excised from the gel. M13 and the *Tne* polymerase gene fragment were ligated together. The products of the ligations were analyzed by agarose gel electrophoresis, and used to transform *E. coli*. This activity was recorded on pages 15-16 of notebook 3966. A copy is attached as Exhibit P-5.

On or about February 23, 1995, Roger Lasken began an experiment to ascertain the properties of various thermostable polymerases in applications such as sequencing, mutagenesis or PCR. In this experiment he performed mismatch extension assays on *Taq*, *Tfl*, *Tne*, Vent®, DeepVent®, and Pfu polymerases. This experiment was recorded on page 138 of notebook 3902. A copy thereof is attached as Exhibit L-46.

On or about February 24, 1995, Roger Lasken continued his experiment to ascertain the properties of various thermostable polymerases in applications such as sequencing, mutagenesis or PCR by analyzing data from the primer degradation assays performed on February 21, 1995. Additionally, he ran mismatch extension assays on *Taq*, *Tfl*, *Tne*, Vent®, DeepVent®, and *Pfu* polymerases. This experiment was recorded on page 139-141 of notebook 3902. A copy thereof is attached as Exhibit L-47.

On or about February 27, 1995, Roger Lasken continued his experiment to ascertain the properties of various thermostable polymerases in applications such as sequencing, mutagenesis or PCR by conducting mismatch repair assays on *Taq*, *Tfl*, *Tne*, Vent®, DeepVent® and *Pfu* polymerases. This experiment was recorded on pages 142-143 of notebook 3902. A copy thereof is attached as Exhibit L-48.

On or about February 28, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* gene fragment into M13. The identities of pTrc/*T.nea*, pSport/*Tne*, M13mp19, and mp19 were confirmed by restriction mapping. This experiment was recorded on pages 20-21 of notebook 3966. A copy thereof is attached as Exhibit P-6.

On or about March 1, 1995, Roger Lasken performed an experiment to determine the effects of potassium acetate on the exonuclease activity of *Tne* and *Taq* polymerases. This experiment was recorded on pages 146-147 of notebook 3902. A copy thereof is attached as Exhibit L-49.

On or about March 1, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* gene fragment into M13. Restriction analysis was performed on M13 DNA and the clones that were transformed on February 23, 1995, to confirm the identities of the constructs. This activity was recorded on pages 22-23 of notebook 3966. A copy thereof is attached as Exhibit P-7.

On or about March 2, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* gene fragment into M13. The products of the restriction reactions performed on March 1, 1995, were purified by agarose gel electrophoresis, and the appropriate fragments were excised from the agarose gel and purified. The excised *Tne* polymerase gene fragment was ligated into M13 and host cells were transformed. This experiment was recorded on pages 23-24 of notebook 3966. A copy thereof is attached as Exhibit P-8.

On or about March 3, 1995, Roger Lasken performed an experiment to determine the effect of enzyme concentration on primer extension activity. These experiments were recorded on page 148-149 of notebook 3902. A copy thereof is attached as Exhibit L-50.

On or about March 4, 1995, Roger Lasken analyzed the data from the *Tne* primer extension experiments performed on March 3, 1995. This analysis was recorded on page 150-151 of notebook 3902. A copy thereof is attached as Exhibit L-51.

On or about March 7, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the experiment to clone a *Tne* gene fragment into M13. The new M13-*Tne* construct ligated on March 2, 1995 was purified, and the structure of the new construct was confirmed by restriction mapping. This experiment was recorded on pages 25-27 of notebook 3966. A copy thereof is attached as Exhibit P-9.

On or about March 8, 1995, Avani Patel, working under supervision by me and Brian Schmidt, performed an experiment to mutate *Tne* DNA polymerase. Site directed mutagenesis reactions were performed on the new *Tne*-M13 construct to generate a 3'-5' exonuclease mutant using the 2899 oligonuceotide primers, which were designed by Brian Schmidt (*see* Exhibit S-7). Additionally attempts were made to ligate the *Tne* polymerase fragment into Mp18 and Mp19; both new constructs were used to transform *E. coli* host cells. This experiment was recorded on pages 28-30 of notebook 3966. A copy thereof is attached as Exhibit P-10.

On or about March 9-13, 1995, Roger Lasken performed experiments to determine the stability of a *Taq* polymerase sample after 33 days of storage in PCR mix (original sample prepared February 2, 1995). This experiment was recorded on pages 152-153 of notebook 3902. A copy thereof is attached as Exhibit L-52.

On or about March 14, 1995, I obtained cells from Brian Schmidt which contained the M13-*Tne* construct. I isolated the ssDNA from CJ236 and purified it on an agarose gel. Additionally, I outlined my ideas for generating F-Y and 3'-5' exonuclease (D to A) mutations

in *Tne* polymerase. I also designed the oligonucleotides needed for the mutagenesis. This activity was recorded on page 23 of notebook 3964. A copy thereof is attached as Exhibit C-1.

On or about March 14, 1995, Avani Patel, working under supervision by me and Brian Schmidt, performed an experiment to culture *Tne*-Mp19 clones. Samples were analyzed by restriction mapping to confirm the identity of the construct. This experiment was recorded on page 31 of notebook 3966. A copy thereof is attached as Exhibit P-11.

On or about March 15, 1995, Roger Lasken performed experiments to determine the optimal conditions for PCR reactions with *Taq* polymerase. In this experiment he noted that a 1/600 dilution of *Taq* polymerase should be used in PCR reactions between 20 and 40 minutes after diluting. This experiment was recorded on pages 155-156 of notebook 3902. A copy thereof is attached as Exhibit L-53.

On or about March 15, 1995, Avani Patel, working under supervision by me and Brian Schmidt, conducted an experiment to analyze the fragment pattern from the restriction mapping experiments performed on March 14, 1995. The *Tne*-M13 clone was grown and cells were pelleted and stored at -70° C overnight. This experiment was recorded on pages 32-33 of notebook 3966. A copy thereof is attached as Exhibit P-12.

On or about March 16, 1995, Avani Patel, working under supervision by me and Brian Schmidt, continued the previous experiment by purifying the vector from the cells stored on March 15, 1995, and restricted the plasmid for further confirmation of the construct. This

experiment was recorded on pages 34-35 of notebook 3966. A copy thereof is attached as Exhibit P-13.

On or about March 20, 1995, Roger Lasken prepared tetracycline stocks and tetracycline plates for use in growing cells for *Tfl* polymerase expression and purification. This experiment was recorded on page 157 of notebook 3902. A copy thereof is attached as Exhibit L-54.

On or about March 21, 1995, Roger Lasken conducted an experiment to begin the process of purifying recombinant *Tfl* polymerase by inoculating cells containing the *Tfl* polymerase gene to amp and tet plates. This experiment was recorded on page 157 of notebook 3902. A copy thereof is attached as Exhibit L-55.

On or about March 22, 1995, Roger Lasken continued the experiment to purify recombinant *Tfl* polymerase by inoculating the *Tfl* clones to liquid media. Cells were grown and expression was induced. Following induction, cells were grown for an additional 15 hours. This experiment was recorded on pages 157-158 of notebook 3902. A copy thereof is attached as Exhibit L-56.

On or about March 23, 1995, Roger Lasken continued the experiment to purify recombinant *Tfl* polymerase. The cells grown on March 22, 1995, were lysed and the lysate was heat treated. Only low levels of polymerase activity were detected in the lysate after heat treatment, indicating the presence of only low levels of *Tfl* DNA polymerase. This experiment was recorded on pages 158-159 of notebook 3902. A copy thereof is attached as Exhibit L-57.



On or about March 24, 1995, Roger Lasken prepared a new PEI stock solutions to be used in the purification of *Tfl* polymerase. This experiment was recorded on page 160 of notebook 3902. A copy thereof is attached as Exhibit L-58.

On or about March 26-27, 1995, Roger Lasken began a new experiment to express and purify recombinant *Tfl* polymerase. He prepared new LB media and ampicillin stocks, into which the *Tfl*-106 clone was inoculated and grown. Expression was induced, and cells were grown for an additional hour. Cells were harvested and frozen at -70° C. This experiment was recorded on page 161 of notebook 3902. A copy thereof is attached as Exhibit L-59.

On or about March 28, 1995, Roger Lasken continued his experiment to express and purify recombinant *Tfl* polymerase by preparing new buffers. This experiment was recorded on page 162 of notebook 3902. A copy thereof is attached as Exhibit L-60.

On or about March 29, 1995, Elizabeth Flynn began an experiment to purify a new 3' exonuclease mutant of *Tne* polymerase. In this experiment she lysed cells containing the 764-D1-00-1R wild-type *Tne* polymerase. The lysate was heat treated and PEI and ammonium sulfate precipitations were performed to purify the polymerase. This activity was recorded on page 108 of notebook 3865. A copy thereof is attached as Exhibit F-3.

On or about March 29, 1995, I performed an experiment to synthesize the second strand for both the 3' exonuclease and FY mutants of *Tne* polymerase using the primers described on March 14, 1995. The new constructs were used to transform *E. coli* host cells. This experiment

was recorded on or about page 24 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-2.

On or about March 29, 1995, Roger Lasken continued the experiment directed to the isolation of recombinant *Tfl* polymerase. He lysed the cells which were grown on March 26, 1995. The lysate was heat treated and PEI and ammonium sulfate precipitations were performed. This experiment was recorded on page 163 of notebook 3902. A copy thereof is attached as Exhibit L-61.

On or about March 30, 1995, Elizabeth Flynn continued the experiment to isolate the 3' exonuclease mutant of *Tne* polymerase. She resuspended the pellet from the ammonium sulfate precipitate obtained on March 29, 1995. The resuspended pellet was dialyzed for 8 hours. Finally, the dialysate was loaded onto a heparin column to purify the polymerase. This experiment was recorded on pages 109-110 of notebook 3865. A copy thereof is attached as Exhibit F-4.

On or about March 30, 1995, Roger Lasken continued the experiment to purify *Tfl* polymerase. In this experiment he performed polymerase assays on the supernatants from the ammonium sulfate precipitation performed on March 29, 1995. This activity was recorded on page 164-165 of notebook 3902. A copy thereof is attached as Exhibit L-62.

On or about March 31, 1995, Elizabeth Flynn continued the experiment to isolate the 3' exonuclease mutant of *Tne* polymerase. She assayed fractions from the heparin column for

polymerase The fractions were analyzed by SDS-PAGE, and Bradford protein concentration assays were performed. This experiment was recorded on pages 111-113 of notebook 3865. A copy thereof is attached as Exhibit F-5.

On or about March 31, 1995, I checked the plates inoculated on March 29, 1995 for the presence of plaques. FY mutant plaques were observed. The presence of the FY mutation was confirmed by restriction digestion. No 3' exonuclease mutant plaques were observed. Therefore, additional host cells were transformed with the 3' exonuclease mutant construct. This experiment was recorded on pages 25-26 of notebook 3964. A copy thereof is attached as Exhibit C-3.

On or about March 31, 1995, Roger Lasken continued the experiment to purify *Tfl* polymerase. He poured a Sephacryl 200 column for further use in *Tfl* isolation. This activity was recorded on page 166 of notebook 3902. A copy thereof is attached as Exhibit L-63.

On or about April 2, 1995, Elizabeth Flynn performed an experiment to isolate *Tne* wild-type 764-D1-00-1R on a Q650 column. This activity was recorded on page 114 of notebook 3865. A copy thereof is attached as Exhibit F-6.

On or about April 3, 1995, Elizabeth Flynn continued the experiment to isolate a *Tne* polymerase mutant by assaying fractions from the Q650 column for polymerase activity. She also assayed the protein concentration of the fractions. This experiment was recorded on pages 115-117 of notebook 3865. A copy thereof is attached as Exhibit F-7.

On or about April 4, 1995, Elizabeth Flynn continued the experiment to isolate a *Tne* polymerase mutant by performing calculations to determine the total activities in the fractions obtained from the Q650 column. These calculations were recorded on page 118 of notebook 3865. A copy thereof is attached as Exhibit F-8.

On or about April 4, 1995, Roger Lasken performed an experiment to test the stability of a sample of *Taq* polymerase which had been stored since February 3, 1995. This experiment was recorded on page 167-169 of notebook 3902. A copy thereof is attached as Exhibit L-64.

On or about April 5, 1995, Elizabeth Flynn continued the experiment to isolate a *Tne* polymerase mutant by re-assaying the total activities of the purified samples obtained from the Q650 column. She performed calculations to determine the percent yield and analyzed the samples by SDS-PAGE. Fractions were also assayed for RNase activity. This activity was recorded on pages 119-121 of notebook 3865. A copy thereof is attached as Exhibit F-9.

On or about April 5, 1995, Roger Lasken began an experiment to purify *Tfl* polymerase by harvesting a new batch of recombinant *Tfl* polymerase-containing cells. The cells were lysed and heat treated. The heat stable polymerase activity was determined after heat treatment. This experiment was recorded on page 170 of notebook 3902. A copy thereof is attached as Exhibit L-65.

On or about April 6, 1995, Elizabeth Flynn continued the experiment to isolate *Tne* polymerase by continuing RNase assays on the newly purified *Tne* mutant. Additionally, she

performed exonuclease assays on the *Tne* mutant fractions. This experiment was recorded on pages 122-125 of notebook 3865. A copy thereof is attached as Exhibit F-10.

On or about April 7, 1995, Roger Lasken continued the experiment to purify the *Tfl* polymerase by culturing additional cells containing *Tfl* polymerase. Cells were grown, induced, harvested, lysed and heat treated. Sodium chloride was added to the lysate and PEI and ammonium sulfate precipitations were performed. This experiment was recorded on pages 171-172 of notebook 3902. A copy thereof is attached as Exhibit L-66.

On or about April 8, 1995, Roger Lasken continued the experiment to purify the *Tfl* polymerase by performing ammonium sulphate precipitation experiments. Since he was unable to get the ammonium sulfate pellet to sediment; the purification experiment was not continued. This experiment was recorded on page 173 of notebook 3902. A copy thereof is attached as Exhibit L-67.

On or about April 11, 1995, Roger Lasken performed an experiment to investigate the stability of *Taq* polymerase. A sample of *Taq* polymerase that had been stored at room temperature for 29 days was assayed for polymerase activity. This activity was recorded on pages 174-175 of notebook 3902. A copy thereof is attached as Exhibit L-68.

On or about April 13, 1995, Roger Lasken began a new experiment to purify *Tfl* polymerase by culturing cells containing the *Tfl* polymerase gene. Cells were harvested and

lysed. Sodium chloride was added to the lysate. This activity was recorded on page 176 of notebook 3902. A copy thereof is attached as Exhibit L-69.

On or about April 15, 1995, I began an experiment to generate the 3'-5' exonuclease mutation in *Tne* polymerase again. I synthesized the second strand using mutagenic primer #2899 obtained from Brian Schmidt (*see* Exhibit S-7), and host cells with the new plasmids containing the 3'-5' exonuclease mutation in the *Tne* polymerase gene. This experiment was recorded on page 29 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-4.

On or about April 17, 1995, Roger Lasken continued the experiment to purify *Tfl* polymerase by performing PEI and ammonium sulfate precipitations on the lysates generated on April 13, 1995. This experiment was recorded on page 117 of notebook 3902. A copy thereof is attached as Exhibit L-70.

On or about April 18, 1995, Roger Lasken continued the experiment to purify *Tfl* polymerase by applying a *Tfl* lysate to a Sephacryl 200 column and obtaining column fractions. Unit concentration assays were performed on the column fractions. This experiment was recorded on pages 178 and 180-181 of notebook 3902. A copy thereof is attached as Exhibit L-71.

On or about April 19, 1995, Roger Lasken continued the experiment to purify *Tfl* polymerase by applying fractions isolated from the S200 column to a Blue Sepharose column.

This experiment was recorded on pages 182 of notebook 3902. A copy thereof is attached as Exhibit L-72.

On or about April 20, 1995, Roger Lasken continued the experiment to purify the *Tfl* polymerase by dialyzing fractions obtained from the Blue Sepharose column. The dialysate was then applied to a heparin column. This experimental activity was recorded on page 183 and 185 of notebook 3902. A copy thereof is attached as Exhibit L-73.

On or about April 21, 1995, I continued an experiment to generate the 3'-5' exonuclease mutation in *Tne* polymerase by isolating the DNA from the host cells that I transformed on April 15, 1995. I analyzed this DNA by restriction analysis to determine which clones contained the mutation. Clones with the correct restriction pattern were saved. This experimental activity was recorded on page 30 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-5.

On or about April 21, 1995, Roger Lasken continued the experiment to purify the *Tfl* polymerase by performing polymerase activity assays on the fractions from the heparin column. This experimental activity was recorded on pages 187-189 of notebook 3902. A copy thereof is attached as Exhibit L-74.

On or about April 23, 1995, Roger Lasken prepared new tetracycline stock solutions and media. Media was quality controlled. This experimental activity was recorded on page 190 of notebook 3902. A copy thereof is attached as Exhibit L-75.

On or about April 24, 1995, Roger Lasken regenerated the Blue sepharose, heparin and S200 columns used to purify the *Tfl* polymerase for future use. This experimental activity was recorded on page 191 of notebook 3902. A copy thereof is attached as Exhibit L-76.

On or about April 25, 1995, Roger Lasken continued the experiment to purify the *Tfl* polymerase by performing SDS-PAGE on column fractions obtained from a previous experiment. This experimental activity was recorded on page 192 of notebook 3902. A copy thereof is attached as Exhibit L-77.

On or about April 26, 1995, Roger Lasken continued the experiment to purify the *Tfl* polymerase by pooling column fractions obtained from a previous experiment and dialyzed them overnight. This activity was recorded on page 1 of notebook 3903. A copy thereof is attached as Exhibit L-78.

On or about April 27, 1995, Roger Lasken continued the experiment to purify the *Tfl* polymerase by removing the samples from dialysis and running them on a heparin column to resolve two remaining peaks. This experimental activity was recorded on pages 2-3 of notebook 3903. A copy thereof is attached as Exhibit L-79.

On or about April 28, 1995, Roger Lasken began an experiment to characterize purified *Tfl* polymerase fractions by performing SDS-PAGE on the fractions from the heparin column.



This experimental activity was recorded on pages 4-5 of notebook 3903. A copy thereof is attached as Exhibit L-80.

On or about April 29, 1995, Roger Lasken prepared the *Tfl* fractions for storage by preparing new *Taq* polymerase storage buffers, and pooling and dialyzing the *Tfl* polymerase fractions. This experimental activity was recorded on page 6 of notebook 3903. A copy thereof is attached as Exhibit L-81.

On or about April 30, 1995, Roger Lasken stored the newly purified *Tfl* polymerase by mixing the dialyzed fractions with storage buffer and stored them at -20° C. This experimental activity was recorded on page 6 of notebook 3903. A copy thereof is attached as Exhibit L-82.

On or about May 1, 1995, I performed an experiment to clone the gene encoding polymerase 3'-5' exonuclease mutant into pUC19-*Tne* by replacing the wild-type *Tne* fragment. Preparative *SphI/HindIII* digests were performed on pUC*Tne* and M13mp19 mutants. The largest fragments of pUC*Tne* and the smallest fragment of M13mp19 were gel purified, ligated and used to transform DH10B. This experimental activity was recorded on page 31 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-6.

On or about May 2, 1995, Roger Lasken performed an experiment to characterize the purified *Tfl* polymerase by performing unit activity concentration assays. This experimental activity was recorded on pages 7-8 of notebook 3903. A copy thereof is attached as Exhibit L-83.

On or about May 3, 1995, Roger Lasken performed a further experiment to characterize the newly purified *Tfl* polymerase. He assayed the newly purified *Tfl* polymerase for endonuclease activity. This experimental activity was recorded on pages 9-10 of notebook 3903. A copy thereof is attached as Exhibit L-84.

Between about May 3, 1995, and about May 9, 1995, Roger Lasken prepared dilutions of *Taq* polymerase for PCR functional assays. This experimental activity was recorded on page 11 of notebook 3903. A copy thereof is attached as Exhibit L-85.

On or about May 4, 1995, I continued an experiment to clone the gene encoding polymerase 3'-5' exonuclease *Tne* mutant into pUC19-*Tne*. I selected colonies from the transformations performed on March 31, 1995 (FY mutant) and May 1, 1995 (3'-5' mutant). The selected clones were purified and the structure of the construct was confirmed by restriction mapping. This experimental activity was recorded on page 32 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-7.

On or about May 9, 1995, Roger Lasken prepared for experiments comparing the properties of *Tfl* and *Tne* polymerases by labeling oligonucleotides for use in the fidelity assays. He also outlined conditions for assaying fidelity of polymerases with and without 3' exonuclease activity. Additionally, he performed unit activity concentration assays on *Tne* and *Tfl* polymerases. This activity was recorded on pages 12-13 of notebook 3903. A copy thereof is attached as Exhibit L-86.

On or about May 10, 1995, I restricted nucleic acids encoding the ptrc99 clone, 3'-5' exo<sup>-</sup> *Tne* mutant, and the FY *Tne* mutant with *Sst*I and *Hind*III. Fragments from each clone were ligated into ptrc99 and transformed into DH10B. This experimental activity was recorded on page 33 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-8.

On or about May 10, 1995, Roger Lasken conducted an experiment to compare the properties of *Tne* and *Tfl* polymerases. He performed 3' exonuclease assays on *Tne* and *Tfl* polymerases in a number of buffer conditions. This experimental activity was recorded on pages 14-15 of notebook 3903. A copy thereof is attached as Exhibit L-87.

On or about May 12, 1995, Roger Lasken conducted an experiment to continue characterizing the newly purified *Tfl* polymerase by assaying for 3' exonuclease activity. He noted that there was no 3' exonuclease activity detected. He also changed the *Tfl* storage conditions from *Taq* storage conditions to Epicenter *Tfl* polymerase conditions. This experimental activity was recorded on pages 16-19 of notebook 3903. A copy thereof is attached as Exhibit L-88.

On or about May 16, 1995, Roger Lasken conducted an experiment to continue characterizing the newly purified *Tfl* polymerase. He performed polymerase assays on mixtures of *Tfl* and Vent® polymerases at various buffer conditions. This experimental activity is recorded on pages 20-21 of notebook 3903. A copy thereof is attached as Exhibit L-89.

On or about May 17, 1995, Roger Lasken or Elizabeth Flynn conducted a unit assay on the *Tne* polymerase preparation from May 7, 1995. This experimental activity was recorded on page 22 of notebook 3903. A copy thereof is attached as Exhibit L-90.

On or about May 18, 1995, Roger Lasken conducted an experiment to characterize fractions from the S200 column by assaying for polymerase activity. This experimental activity was recorded on pages 24-25 of notebook 3903. A copy thereof is attached as Exhibit L-91.

On or about May 19, 1995, Roger Lasken conducted an experiment to continue characterizing the *Tne* polymerase by performing unit concentration assays on the fractions from the S200 column. This activity was recorded on page 25 of notebook 3903. A copy thereof is attached as Exhibit L-92.

On or about May 22, 1995, Elizabeth Flynn conducted an experiment to begin purification of the 3' exonuclease mutant of *Tne* polymerase. She lysed host cells transformed with DNA encoding the 3'-5' exonuclease mutant of *Tne* polymerase. Additionally a heparin column was equilibrated. This experimental activity was recorded on page 129 of notebook 3865. A copy thereof is attached as Exhibit F-11.

On or about May 22, 1995, Roger Lasken conducted an experiment to continue characterizing the properties of *Tfl* polymerase by performing 3' exonuclease activity assays on a mixture of *Tfl* and Vent® polymerases. This experimental activity was recorded on page 26 of notebook 3903. A copy thereof is attached as Exhibit L-93.

On or about May 23, 1995, Roger Lasken conducted an experiment to continue characterizing the properties of *Tfl* polymerase by performing 3' exonuclease activity assays on a mixture of *Tfl* and Vent® polymerases. This experimental activity was recorded on pages 27-28 of notebook 3903. A copy thereof is attached as Exhibit L-94.

On or about May 24, 1995, Roger Lasken analyzed data from the 3' exonuclease assays on mixtures of *Tfl* and Vent® polymerases. He also performed unit concentration assays on *Tfl*/Vent® polymerase mixtures prepared by Nin Guan, another LTI employee. Additionally, he performed long PCR experiments to measure the turnover of mixtures of *Tfl* and Vent® polymerases. This activity was recorded on pages 29-31 of notebook 3903. A copy thereof is attached as Exhibit L-95.

On or about May 25, 1995, Elizabeth Flynn performed an experiment to purify the 3' exonuclease mutant of *The* polymerase on a heparin column. Polymerase activities of the heparin column fractions were assayed. The fractions were heat treated. This experimental activity was recorded on pages 130-131 of notebook 3865. A copy thereof is attached as Exhibit F-12.

On or about May 25, 1995, Roger Lasken performed unit concentration assays 1.1x *rTaq*, old *rTaq* (1.1x), new *rTaq* (1.1x) and BM2X polymerase mixtures. This experimental activity is recorded on pages 34-35 of notebook 3903. A copy thereof is attached as Exhibit L-96.

On or about May 26, 1995, Roger Lasken analyzed the data collected in the unit activity concentration assays performed on May 25, 1995. This analysis is recorded on page 36 of notebook 3903. A copy thereof is attached as Exhibit L-97.

On or about May 30, 1995, Roger Lasken performed unit activity concentration assays to determine the stability of 1.1x *Taq* polymerase stored at room temperature in reaction mixture. This experimental activity was recorded on pages 37-39 of notebook 3903. A copy thereof is attached as Exhibit L-98.

On or about May 31, 1995, Roger Lasken and Carolyn Combs performed experiments directed to determining the stability of thermostable polymerases. In this experiment they assayed the stability of *Tfl* and Vent® polymerases at 68° C over 20 minutes. This experimental activity was recorded on pages 40-42 of notebook 3903. A copy thereof is attached as Exhibit L-99.

On or about May 31, 1995, Roger Lasken prepared new 5 unit/ $\mu$ l dilutions of r*Taq* polymerase, lot EKB1. This experimental activity is recorded on page 43 of notebook 3903. A copy thereof is attached as Exhibit L-100.

On or about June 2, 1995, Roger Lasken further characterized the *Tfl* polymerase by performing primer degradation assays using mixtures of *Tfl* and Vent® polymerase, and saw no apparent endonuclease or 5' exonuclease activity in the *Tfl* samples. This activity was recorded on pages 44-46 of notebook 3903. A copy thereof is attached as Exhibit L-101.

On or about June 5, 1995, Roger Lasken and Carolyn Combs performed an experiment to assay mixtures of *Tfl* and Vent® polymerases for 3' exonuclease activity using the primer degradation assay. This experimental activity was recorded on pages 1-2 of notebook 4092. A copy thereof is attached as Exhibit L-102.

On or about June 6, 1995, Roger Lasken and Carolyn Combs performed an experiment to assay mixtures of *Tfl* and Vent® polymerases for 3' exonuclease activity using the primer degradation assay. Additionally they determined the mobility of a full length <sup>32</sup>P labeled 33-mer on a PEI plate. This experimental activity was recorded on pages 3-4 and 7-8 of notebook 4092. A copy thereof is attached as Exhibit L-103.

On or about June 7, 1995, Roger Lasken and Carolyn Combs performed an experiment to measure the 3' exonuclease activity in mixtures of *Tfl* and Vent® polymerases in 3 different buffer conditions to establish a baseline for the stability studies. This experimental activity was recorded on pages 9-10 of notebook 4092. A copy thereof is attached as Exhibit L-104.

On or about June 8, 1995, I obtained colonies from the transformation performed on May 5, 1995, and attempted to confirm the identity of the resulting clones using a restriction digest analysis. This experimental activity was recorded on page 34 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-9.

On or about June 9, 1995, I performed an experiment to test 8 more proposed FY mutant clones using restriction digest analysis. Additionally I inoculated 1 liter of ptrc*Tne35* for

induction and purification. This experimental activity was recorded on page 35 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-10.

On or about June 9, 1995, Roger Lasken performed unit assays on Nin Guan's mixtures of *Tfl* and Vent® polymerases using various dilutions. Additionally he attempted to optimize the assay's signal to noise ratio and linearity. This experimental activity was recorded on pages 48-49 of notebook 3903. A copy thereof is attached as Exhibit L-105.

On or about June 10, 1995, I performed an experiment to obtain both the 3' exonuclease mutation and the FY mutation in the same *Tne* polymerase gene. pUC 3' exonuclease and FY mutant clones were restricted with *Bam*HI. Fragments were gel purified and the largest 3' exonuclease mutant fragment was ligated to the smallest FY mutant fragment. The new construct was used to transform *E. coli* DH10B. This experimental activity was recorded on page 36 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-11.

On or about June 12, 1995, Roger Lasken and Carolyn Combs performed a quality control analysis on a new batch of PEI plates. Plates will be used for the *Tfl*/Vent® polymerase turnover experiments. This experimental activity was recorded on page 13 of notebook 4092. A copy thereof is attached as Exhibit L-106.

On or about June 13, 1995, Elizabeth Flynn performed an experiment to determine the amount of heat stable polymerase activity in the crude lysate containing *Tne* 3' exonuclease



mutants. This experimental activity was recorded on pages 132-133 of notebook 3865. A copy thereof is attached as Exhibit F-13.

On or about June 13, 1995, Roger Lasken performed an experiment to further characterize *Tfl* polymerase. He repeated exonuclease assays on *Tfl* polymerase using new primers. This experimental activity is recorded on pages 50-51 of notebook 3903. A copy thereof is attached as Exhibit L-107.

On or about June 13, 1995, Roger Lasken and Carolyn Combs performed an experiment to continue their characterization of *Tfl* polymerase. In this experiment they assayed both the turnover and incorporation rates of a mixture of *Tfl* and Vent® polymerases at varying concentrations of *Tfl* polymerase. This experimental activity was recorded on pages 17-18 of notebook 4092. A copy thereof is attached as Exhibit L-108.

On or about June 14, 1995, Elizabeth Flynn performed an experiment to reassay the crude lysates obtained from bacteria transformed with the new *Tne* 3' exonuclease mutant and from bacteria transformed with the new FY *Tne* mutant for thermostable polymerase activity. This experimental activity was recorded on pages 134-138 of notebook 3865. A copy thereof is attached as Exhibit F-14.

On or about June 14, 1995, Roger Lasken and Carolyn Combs further analyzed the data from the incorporation experiments performed on June 13, 1995. Additionally, they more accurately determined the turnover and incorporation rates of mixtures of *Tfl* and Vent®

polymerases for use in the *Tfl*/Vent® stability studies. This activity is recorded on page 20-23 of notebook 4092. A copy thereof is attached as Exhibit L-109.

On or about June 15, 1995, Elizabeth Flynn performed an experiment to continue purification of the 3' exonuclease mutants of *Tfl* polymerase. She lysed new batches of cells containing the FY mutant and the 3' exonuclease mutant, performed PEI and ammonium sulfate precipitations on the lysates, and applied the FY and the 3' exonuclease mutant lysates to a heparin column. Fractions obtained from the heparin columns were assayed for polymerase activity. This experimental activity was recorded on pages 139-143 of notebook 3865. A copy thereof is attached as Exhibit F-15.

On or about June 15, 1995, I performed an experiment to analyze clones obtained from the transformation performed on June 10, 1995, using restriction digest mapping. Clones with the correct restriction patterns were identified and further tested for the presence of an *Eco*47III site. This experimental activity was recorded on page 38 of notebook 3964. A true and accurate copy thereof is attached as Exhibit C-12.

On or about June 15, 1995, Roger Lasken and Carolyn Combs performed an experiment to continue the characterization of *Tfl* polymerase. They analyzed data from experiments performed on June 14, 1995. This activity is recorded on page 24 of notebook 4092. A copy thereof is attached as Exhibit L-110.

On or about June 16, 1995, Elizabeth Flynn performed an experiment to continue purification of the 3' exonuclease and FY mutants of *Tne* polymerase. On this date she loaded fractions containing the FY *Tne* mutant and the 3' exonuclease *Tne* mutant on a Q650 column. Fractions obtained from the Q650 column were dialyzed overnight. This activity was recorded on pages 146-148 of notebook 3865. A copy thereof is attached as Exhibit F-16.

On or about June 19, 1995, Elizabeth Flynn continued work toward purifying the 3' exonuclease mutant of *Tne* polymerase by removing the samples from the experiment performed June 16, 1995 (*see* Exhibit F-16), from dialysis. This experimental activity is recorded on page 149 of notebook 3865. A copy thereof is attached at Exhibit F-17.

On or about June 19, 1995, Roger Lasken and Carolyn Combs performed a further experiment to analyze turnover data from the experiments performed on June 13, 1995. Additionally, they determined the optimal running time for the PEI plates to minimize background when detecting  $^{32}\text{P}$ -dATP. This experimental activity was recorded on page 19 of notebook 4092. A copy thereof is attached as Exhibit L-111.

On or about June 20, 1995, Roger Lasken and Carolyn Combs analyzed data from the optimization experiments performed on June 19, 1995, and determined that errors occurred during the experiments. This activity was recorded on page 30 of notebook 4092. A copy thereof is attached as Exhibit L-112.

On or about June 21, 1995, Roger Lasken and Carolyn Combs performed primer degradation assays on mixtures of *Tfl* and Vent® polymerases, as well as Vent® polymerase alone. This experimental activity was recorded on pages 33-36 of notebook 4092. A copy thereof is attached as Exhibit L-113.

On or about June 23, 1995, Roger Lasken began experiments directed at the optimization of the M13 PCR system, which would be used in subsequent experiments on thermostable polymerases and their uses. On this date he performed experiments using *Tne* and *Taq* polymerases in the M13 PCR system at varying reaction conditions in an attempt to optimize the reaction buffer conditions. This activity was recorded on pages 40-41 of notebook 4092. A copy thereof is attached as Exhibit L-114.

On or about June 24, 1995, Roger Lasken and Carolyn Combs further analyzed data and wrote conclusions based on the incorporation and turnover assays performed on June 14, 1995, and June 19, 1995. They determined that in order to optimally resolve the dATP/dADP peak from the dAMP peak, the LiCl solvent should be run to the top of the PEI plate. This activity was recorded on pages 25, 31 and 42 of notebook 4092. A copy thereof is attached as Exhibit L-115.

On or about June 26, 1995, Roger Lasken and Carolyn Combs performed experiments to optimize the M13 PCR system. In these experiments she diluted primers for use in the M13 PCR experiments to working concentrations, and ran agarose gels of the reaction products. Additionally the annealing temperature, *Taq* concentration, cycle number, and primer

concentrations was optimized for the M13 PCR system. This experimental activity was recorded on pages 43-45 and 47-51 of notebook 4092. A copy thereof is attached as Exhibit L-116.

On or about June 27, 1995, Elizabeth Flynn performed an experiment to determine the total activity of the purified fractions of the *Tne* 3' exonuclease mutant. This experimental activity was recorded on pages 150-151 of notebook 3865. A copy thereof is attached as F-18.

On or about June 28, 1995, Roger Lasken performed an experiment to determine the stability of *Taq* polymerase when stored in reaction mix. Samples were analyzed which had been stored at 4° C, -20° C and -70° C for 0-5 months. This experimental activity was recorded on page 52 of notebook 3903. A copy thereof is attached as Exhibit L-117.

On or about June 29, 1995, Roger Lasken analyzed data from the *Taq* stability assays performed on June 28, 1995. This activity was recorded on page 53 of notebook 3903. A copy thereof is attached as Exhibit L-118.

On or about June 29, 1995, Roger Lasken and Carolyn Combs performed an experiment to continue the characterization of *Tfl* polymerase. They performed 3' exonuclease turnover assays on mixtures of *Tfl* and Vent® polymerases as well as Epicenter *Tfl*/Vent® polymerase mixture. Additionally, they optimized primer and enzyme concentrations in the M13 PCR system, and prepared Mp19 ssDNA as a substrate for the 3' exonuclease activity assays. This experimental activity was recorded on pages 53-54 and 58-63 of notebook 4092. A copy thereof is attached as Exhibit L-119.

On or about June 30, 1995, Roger Lasken performed an experiment continuing his analysis of *Taq* stability when stored in reaction buffer. This activity was recorded on page 53 of notebook 3903. A copy thereof is attached as Exhibit L-120.

On or about June 30, 1995, Carolyn Combs, working under supervision by Roger Lasken, continued the characterization of *Tfl* polymerase by analyzing data from the turnover experiments performed on June 29, 1995. This analysis was recorded on page 55 of notebook 4092. A copy thereof is attached as Exhibit L-121.

On or about June 30, 1995, Roger Lasken and Carolyn Combs performed 3' exonuclease turnover assays using mixtures of *Tfl* and Vent® polymerases with Mp 19 ssDNA as a template. This activity was recorded on page 64 of notebook 4092. A copy thereof is attached as Exhibit L-122.

On or about July 1, 1995, Roger Lasken and Carolyn Combs continued the characterization of the *Tfl* polymerase by analyzing data from the 3' exonuclease turnover assays performed on June 30, 1995. This activity was recorded on pages 66-67 of notebook 4092. A copy thereof is attached as Exhibit L-123.

On or about July 5, 1995, Roger Lasken and Carolyn Combs performed a further experiment to optimize primer concentration, annealing temperature, and template concentration for the M13 PCR system. This activity was recorded on pages 69-70 of notebook 4092. A copy thereof is attached as Exhibit L-124.

On or about July 6, 1995, Roger Lasken and Carolyn Combs optimized annealing temperature, template concentration, and enzyme concentration in the M13 PCR system using *Tne* and *Taq* polymerases. This activity was recorded on pages 71-76 of notebook 4092. A copy thereof is attached to as Exhibit L-125.

On or about July 7, 1995, Roger Lasken and Carolyn Combs continued the experiment to further optimize the enzyme concentrations used in the M13 PCR system. This activity was recorded on page 77 of notebook 4092. A copy thereof is attached as Exhibit L-126.

On or about July 10, 1995, Roger Lasken and Carolyn Combs performed reactions comparing the activities of old and new samples of *Tne* polymerase in the M13 PCR system. This activity was recorded on page 78 of notebook 4092. A copy thereof is attached as Exhibit L-127.

On or about July 11, 1995, Carolyn Combs, working under supervision by Roger Lasken, continued characterization of the *Tfl* polymerase by generating additional graphs from the turnover experiment data obtained on June 29, 1995. This activity was recorded on page 57 of notebook 4092. A copy thereof is attached as Exhibit L-128.

On or about July 11, 1995, Roger Lasken and Carolyn Combs further optimized the M13 PCR assay system by optimizing enzyme concentration when Cheng buffer is used. This activity was recorded on page 81 of notebook 4092. A copy thereof is attached as Exhibit L-129.

On or about July 12, 1995, Mary Longo began a new project aimed at cloning nucleic acid encoding a *Tne* triple mutant lacking 3'-5' exonuclease activity, 5'-3' exonuclease activity, and the Phe to Tyr substitution in the O-helix into the plasmid ptrc99A. She restricted pUCTne35FY (clone #1) with BspHI and restricted ptrc99A with NcoI and HindIII. The 4.1 kb band from the pUCTne35FY mutant was cut out of the gel and frozen at -20° C overnight. This activity was recorded on page 157 of notebook 3959. A copy thereof is attached as Exhibit M-1.

On or about July 12, 1995, Roger Lasken and Carolyn Combs began experiments characterizing the properties of *Tne* polymerase. On this date they performed an experiment to determine if *Tne* polymerase can produce the 3 smallest M13 PCR fragments in the M13 PCR assay system. This activity was recorded on page 84 of notebook 4092. A copy thereof is attached as Exhibit L-130.

On or about July 13, 1995, Mary Longo continued her efforts to clone the 35FY mutant of *Tne* polymerase. In this experiment she ethanol precipitated the restriction digests performed on July 12, 1995 and dissolved the products in TE buffer. The restriction products were further restricted with BspHI. The vector (ptrc99) and the 1.7 kb BspHI fragment were gel purified and DNA concentrations for both were determined. Clones #1 and #15 of pUCTne35FY were pooled and dissolved in TE buffer. The pooled DNA sample was then restricted with BspHI and the sample was frozen at -20° C overnight. This activity was recorded on page 157 of notebook 3959. A copy thereof is attached as Exhibit M-2.



On or about July 13, 1995, Roger Lasken and Carolyn Combs continued the characterization of *Tfl* polymerase. In this experiment they performed 3' exonuclease turnover assays using *Tfl*/Vent® polymerase mixtures with Mp19 ssDNA as the template. This activity was recorded on pages 87-88 of notebook 4092. A copy thereof is attached as Exhibit L-131.

On or about July 14, 1995, Mary Longo continued her attempts to clone the *Tne* 35FY mutant. In this experiment she ethanol precipitated the pUC35FY restriction product from July 13, 1995, and dissolved it in 1xR2 buffer. The mixture was then restricted with HindIII and the sample was applied to an LMP agarose gel. The 200 bp fragment was excised from the gel and frozen at -20° C. This activity was recorded on page 159 of notebook 3959. A copy thereof is attached as Exhibit M-3.

On or about July 14, 1995, Roger Lasken and Carolyn Combs performed reactions under various conditions to determine which component of Cheng buffer is responsible for a smear seen on gels of PCR reaction products when the reactions were performed using *Tne* polymerase. They also performed other experiments to rule out other causes of the smear. Additionally they ran gels of the optimization reactions performed on July 11, 1995 (*see* Exhibit L-129). This activity was recorded on pages 82-83, 85-86, 90-92 and 94-97 of notebook 4092. A copy thereof is attached as Exhibit L-132.

On or about July 15, 1995, Roger Lasken and Carolyn Combs prepared new stop solutions. This activity was recorded on page 79 of notebook 4092. A copy thereof is attached as Exhibit L-133.

On or about July 18, 1995, Roger Lasken and Carolyn Combs began purification of *Tne* polymerase using a purification strategy similar to that utilized in the *Taq* polymerase purification. New buffers were made, cells were spun, lysed and heat treated. PEI and ammonium sulfate precipitations were performed on the lysate and activities were measured. Additionally, a new strategy for large scale purification of *Tne* polymerase was outlined. New cells were spun, lysed and heat treated, NaCl was added and a PEI precipitation was performed. This activity was recorded on page 100-103 and 105 of notebook 4092. A copy thereof is attached as Exhibit L-134.

On or about July 18, 1995, Mary Longo continued her efforts to clone the *Tne* 35FY mutant. In this experiment she purified the DNA from the July 14, 1995 experiment via phenol-chloroform extraction method, and dissolved it in TE buffer. This activity was recorded on page 159 of notebook 3959. A copy thereof is attached as Exhibit M-4.

On or about July 19, 1995, I cloned both the 3' exonuclease mutation and the FY mutation into pTTQ19 to incorporate both mutations into *Tne* polymerase. This activity was recorded on page 39 of notebook 3964. A copy thereof is attached as Exhibit C-13.

On or about July 19, 1995, Roger Lasken and Carolyn Combs continued the purification of *Tne* polymerase. In this experiment they performed an ammonium sulfate precipitation on the fractions from the PEI precipitation of the large scale *Tne* polymerase preparation. Blue sepharose and S200 columns were equilibrated, and ammonium sulfate fractions were run on an S200 column. This activity was recorded on page 106-107 of notebook 4092. A copy thereof is attached as Exhibit L-135.

On or about July 20, 1995, I continued the cloning of the 3' exonuclease and FY mutations into *Tne* polymerase. The blunt end fragments containing both mutations were restricted with SphI. The fragments were then ligated into pTTQ and transformed into DH10B. This activity was recorded on page 40 of notebook 3964. A copy thereof is attached as Exhibit C-14.

On or about July 20, 1995, Roger Lasken and Carolyn Combs performed experiments in support of their efforts to purify *Tne* polymerase. They performed unit concentration assays on the fractions from the S200 column, and the fractions were run on a Blue sepharose column. This activity was recorded on pages 108-109 of notebook 4092. A copy thereof is attached as Exhibit L-136.

On or about July 21, 1995, I checked the clones from the transformation performed on July 20, 1995 via restriction analysis for the presence of both mutations. This work was recorded on page 41 of notebook 3964. A copy thereof is attached as Exhibit C-15.

On or about July 21, 1995, Roger Lasken and Carolyn Combs performed unit activity concentration assays on the fractions of the large scale *Tne* polymerase preparation that were run on a Blue Sepharose column (*see* Exhibit L-136). The fractions from that column were then dialyzed overnight. This activity was recorded on pages 109-111 of notebook 4092. A copy thereof is attached as Exhibit L-137.

On or about July 22, 1995, Roger Lasken and Carolyn Combs performed unit concentration assays on old and newly purified samples of *Tne* polymerase. A heparin column was loaded with

dialysate from July 21, 1995. Fractions from the heparin column were pooled, dialyzed and unit activity concentration assays were performed. This activity was recorded on pages 112 and 114-117 of notebook 4092. A copy thereof is attached as Exhibit L-138.

On or about July 23, 1995, Roger Lasken and Carolyn Combs used the purified *Tne* polymerase fractions from the Blue sepharose and heparin column to see if they produced a characteristic smear when used in PCR reactions. This activity was recorded on pages 118-120 of notebook 4092. A copy thereof is attached as Exhibit L-139.

On or about July 27, 1995, Roger Lasken and Carolyn Combs began comparisons of *Tne* and *Tal* polymerases in the polymerase chain reaction. In this experiment they optimized PCR conditions using *Tne* and *Taq* polymerases. This activity was recorded on pages 122-127 of notebook 4092. A copy thereof is attached as Exhibit L-140.

On or about July 28, 1995, Roger Lasken continued comparing *Tne* and *Taq* polymerase by assaying *Taq*, *Tne* and Vent® polymerases for ability to extend and incorporate thymidine into a newly synthesized DNA strand from a uracil containing template. This activity was recorded on pages 54 and 56-57 of notebook 3903. A copy thereof is attached as Exhibit L-141.

On or about July 28, 1995, Roger Lasken and Carolyn Combs further investigated the use of *Tne* polymerase on PCR reactions. They determined the effect of annealing temperature on the formation of the smear in PCR reactions with *Tne* polymerase. This activity was recorded on pages 128-129 of notebook 4092. A copy thereof is attached as Exhibit L-142.

On or about July 31, 1995, Roger Lasken performed unit activity concentration assays on 1.1x *Taq* polymerase to be used in future experiments. This activity was recorded on page 60 of notebook 3903. A copy thereof is attached as Exhibit L-143.

On or about July 31, 1995, Roger Lasken and Carolyn Combs compared hot start and cold start PCR using *Tne* polymerase. Additionally they determined that the addition of genomic DNA to the PCR reaction had no effect on the formation of the smear. This activity was recorded on pages 130-132 of notebook 4092. A copy thereof is attached as Exhibit L-144.

On or about August 1, 1995, Roger Lasken and Carolyn Combs determined the effect of low  $Mg^{++}$  concentration on short PCR with *Tne* polymerase. This activity was recorded on pages 133 and 135 of notebook 4092. A copy thereof is attached as Exhibit L-145.

On or about August 1, 1995, Mary Longo began a new scheme for cloning the *Tne35FY* mutant of *Tne* polymerase into ptrc99A or a similar vector. The new scheme involved restricting pUCTne35FY with HindIII, and filling in the ends with Klenow polymerase. This fragment was then restricted with SphI, and the 2 kb fragment was gel purified and then cloned into the SmaI/SphI site of pTTQ19. pTTQ19 was restricted with SmaI, and pUCTne35FY was restricted with HindIII. Restrictions were run on an agarose gel to confirm the band pattern. The pTTQ19-SmaI digest was then subsequently digested with SphI. The pUCTne35FY-HindIII fragment was treated with Klenow polymerase. The fragment was purified by phenol extraction, and

resuspended in 40  $\mu$ l of R6 buffer. The sample was then restricted with SphI and run on an agarose gel. 2kb band was excised from the gel and frozen at -20° C. The fragment was then purified by the GENECLAN method and dissolved in 10  $\mu$ l TE buffer. Concentrations of both the pTTQ19-SmaI-SphI digest, and the 2kb HindIII-klenow-SphI fragment from pUCTne35FY were determined by gel analysis. The two fragments were then ligated together at room temperature for 30 minutes. Jason Potter, another employee of LTI, transformed the ligation into DH10B competent cells, and plated the transformed cells on tet/amp plates. Cells were grown overnight. This activity was recorded on pages 181-183 of notebook 3959. A copy thereof is attached as Exhibit M-5.

On or about August 2, 1995, Mary Longo continued her cloning of the *Tne* 35FY mutant. On this day she picked eight colonies from plates inoculated on August 1, 1995 (*see* Exhibit M-5), and inoculated them to liquid media. This activity was recorded on page 183 of notebook 3959. A copy thereof is attached as Exhibit M-6.

On or about August 2, 1995, Roger Lasken and Carolyn Combs analyzed results of the PCR experiments performed on August 1, 1995. Additionally the effect of Mg<sup>++</sup> concentration on long PCR using *Tne* and *Taq* polymerases was investigated. Oligonucleotides were also radiolabeled for use in future experiments. This activity was recorded on pages 134, 136-138 and 140 of notebook 4092. A copy thereof is attached as Exhibit L-146.

On or about August 3, 1995, Mary Longo continued her efforts to clone the *Tne* 35FY mutant. She isolated plasmid DNA from cells grown on August 2, 1995. Plasmids were restricted

with SphI and EcoRI to confirm correct restriction patterns. This activity was recorded on page 183 of notebook 3959. A copy thereof is attached as Exhibit M-7.

On or about August 3, 1995, Roger Lasken and Carolyn Combs continued experiments in support of their comparisons of *Tne* and *Taq* polymerase. In this experiment they determined the ability of *Taq* polymerase to extend from short primers at varying *Taq* concentrations. This activity was recorded on pages 142-143 and 145 in notebook 4092. A copy thereof is attached as Exhibit L-147.

On or about August 4, 1995, Roger Lasken and Carolyn Combs compared the fidelity of *Taq* and *Tne* polymerases using primers of varying lengths. This activity was recorded on pages 146-147 of notebook 4092. A copy thereof is attached as Exhibit L-148.

On or about August 7, 1995, Roger Lasken and Carolyn Combs analyzed the data from the Mg<sup>++</sup> titration experiments performed on August 2, 1995, and the fidelity experiments performed on August 4, 1995. This activity was recorded on pages 139 and 148-149 of notebook 4092. A copy thereof is attached as Exhibit L-149.

On or about August 8, 1995, Roger Lasken radiolabeled primers for 3' exonuclease assays. This activity was recorded on page 61 of notebook 3903. A copy thereof is attached as Exhibit L-150.

On or about August 8, 1995, Carolyn Combs, working under supervision by Roger Lasken, compared the time course for primer extension of *Taq* and *Tne* polymerases using varying concentrations of enzymes. This activity was recorded on pages 152-153 of notebook 4092. A copy thereof is attached as Exhibit L-151.

On or about August 9, 1995, Roger Lasken began characterization of a 3' exonuclease mutant of the Klenow fragment of *E. coli* polymerase I, obtained from Dr. Catherine Joyce at Yale University (New Haven, CT), by performing 3' exonuclease activity assays to determine the amount of residual activity in the mutant. Additionally he assayed the polymerase activity of r*Taq* polymerase at various dTTP concentrations to determine what the optimal dTTP concentration is for maximum synthesis rate. This activity was recorded on pages 62-66 of notebook 3903. A copy thereof is attached as Exhibit L-152.

On or about August 9, 1995, Carolyn Combs, working under supervision by Roger Lasken, continued the comparison of *Taq* and *Tne* polymerases. In this experiment she performed processivity assays on *Taq*, *Tne* and UITma® polymerases by extension of a 33-mer primer annealed to M13 ssDNA. This activity was recorded on pages 155-156 of notebook 4092. A copy thereof is attached as Exhibit L-153.

On or about August 10, 1995, Carolyn Combs, working under supervision by Roger Lasken, optimized PCR conditions when using a 16-mer oligonucleotide primer with *Tne* and *Taq* polymerases. This activity was recorded on pages 158-162 of notebook 4092. A copy thereof is attached as Exhibit L-154.



On or about August 11, 1995, Roger Lasken continued the comparisons of *Tne* and *Taq* polymerase. On this date he performed processivity assays on *Tne*, *Taq* and Ultima polymerases similar to those performed on August 9, 1995, but at lower polymerase concentrations. This activity was recorded on page 164 of notebook 4092. A copy thereof is attached as Exhibit L-155.

On or about August 14, 1995, Roger Lasken analyzed the data from the exonuclease experiments performed on August 9, 1995, comparing the activities of *Taq* and *Tne* polymerases. Additionally he determined the  $K_m$  of *Taq* polymerase for dTTP in the polymerase assay. This activity was recorded on page 63 of notebook 3903. A copy thereof is attached as Exhibit L-156.

On or about August 15, 1995, Roger Lasken continued his experiments into the stability of thermostable polymerases. In this experiment he optimized the dATP, dGTP and dCTP concentrations in the reaction buffer used for long term storage of *Taq* polymerase. Optimization was based on polymerase activity. This activity was recorded on pages 68-71 of notebook 3903. A copy thereof is attached as Exhibit L-157.

On or about August 15, 1995, Carolyn Combs, working under supervision by Roger Lasken, treated *Tne* polymerase with DNase to determine if genomic DNA contamination is the cause of the smear in PCR reactions using *Tne*. This activity was recorded on pages 166-168 of notebook 4092. A copy thereof is attached as Exhibit L-158.

On or about August 16, 1995, Roger Lasken repeated unit activity concentration assays on 1.1x *Taq* polymerase. This activity was recorded on page 72 of notebook 3903. A copy thereof is attached as Exhibit L-159.

On or about August 16, 1995, Roger Lasken and Carolyn Combs analyzed data from DNase treatment experiments performed on August 15, 1995. This activity was recorded on pages 169-174 of notebook 4092. A copy thereof is attached as Exhibit L-160.

On or about August 17, 1995, Roger Lasken continued the investigation of the stability of thermostable polymerases by comparing the activities of samples of *Taq* polymerases which had been stored at -20° C and 4° C. This activity was recorded on page 73 of notebook 3903. A copy thereof is attached as Exhibit L-161.

On or about August 17, 1995, Carolyn Combs, working under supervision by Roger Lasken, further characterized *Tne* polymerase by performing additional PCR reactions using DNase-treated *Tne* polymerase, and determined that KCl did not inhibit the smear formation. This activity was recorded on page 175 of notebook 4092. A copy thereof is attached as Exhibit L-162.

On or about August 18, 1995, Carolyn Combs, working under supervision by Roger Lasken, continued characterizing *Tne* polymerase. She performed additional PCR reactions using DNase treated *Tne* polymerase. This activity was recorded on pages 176-177 of notebook 4092. A copy thereof is attached as Exhibit L-163.

On or about August 21, 1995, Roger Lasken further characterized the activity of the exonuclease mutant of Klenow fragment by performing strand displacement assays on the exonuclease mutant of Klenow polymerase. This activity was recorded on page 74 of notebook 3903. A copy thereof is attached as Exhibit L-164.

On or about August 21, 1995, Roger Lasken and Carolyn Combs optimized the  $Mg^{++}$  concentration in PCR reactions with *Tne* polymerase. Additionally, they attempted to see if DNase- treated *Tne* will inhibit untreated *Tne* to determine what effect of DNase treatment has on *Tne* activity. This activity was recorded on pages 179-181 of notebook 4092. A copy thereof is attached as Exhibit L-165.

On or about August 22, 1995, Carolyn Combs, working under supervision by Roger Lasken, repeated the attempt to determine if DNase- treated *Tne* polymerase would inhibit untreated *Tne* polymerase. This activity was recorded on pages 182-187 of notebook 4092. A copy thereof is attached as Exhibit L-166.

On or about August 23, 1995, Carolyn Combs, working under supervision by Roger Lasken, analyzed results from experiments involving the smear on the gels of the products of the *Tne* PCR reaction, and conclusions were discussed. This activity was recorded on page 184 of notebook 4092. A copy thereof is attached as Exhibit L-167.

On or about August 24, 1995, Roger Lasken continued attempts to determine the cause of the smear on gels from *Tne* PCR reactions. This activity was recorded on page 75 of notebook 3903. A copy thereof is attached as Exhibit L-168.

On or about August 28, 1995, Roger Lasken repeated his attempt at incorporating  $^{32}\text{P}$  into the smear from the *Tne* PCR reactions. A new sample of *Tne* polymerase was used. This activity was recorded on page 82 of notebook 3903. A copy thereof is attached as Exhibit L-169.

On or about August 29, 1995, Roger Lasken began characterization of the new *Tne* FY mutant. He performed processivity assays on the *Tne* FY mutant as well as UITma® polymerase and Elizabeth Flynn's preparation of 3'-5' exo<sup>-</sup> mutant *Tne* polymerase (see Exhibits F-3 through F-18). This activity was recorded on page 86 of notebook 3903. A copy thereof is attached as Exhibit L-170.

On or about August 31, 1995, Roger Lasken began characterizing *Tne*Δ5FY and comparing its activity to that of other thermostable polymerases. In this experiment he assayed *Taq*, Vent® and *Tne*Δ5FY mutant for the ability to elongate and incorporate thymidines into a new DNA strand from a uracil-containing template. Additionally, he outlined conditions for 3' exonuclease assays on wild-type *Tne*, UITma® and the *Tne*Δ5FY mutant. This activity was recorded on page 88 of notebook 3903. A copy thereof is attached as Exhibit L-171.

On or about September 1, 1995, Roger Lasken further characterized the activity of the *Tne*Δ5FY mutant. He performed 3' exonuclease assays on wild-type *Tne*, UITma® and the *Tne*Δ5FY polymerases. Additionally, he attempted PCR reactions with wild-type *Tne* and the *Tne*Δ5FY mutant to optimize buffer conditions. This activity is recorded on pages 92-93 of notebook 3903. A copy thereof is attached as Exhibit L-172.

On or about September 6, 1995, Roger Lasken investigated the use of the *Tne*Δ5FY mutant in PCR reactions. He optimized conditions for PCR with *Tne*Δ5FY at varying template lengths and

buffer conditions. This activity was recorded on pages 94-95 of notebook 3903. A copy thereof is attached as Exhibit L-173.

On or about September 7, 1995, Roger Lasken continued optimizing PCR conditions with *Tne*Δ5FY mutant. Specifically, salt conditions were optimized. This activity was recorded on pages 96-97 of notebook 3903. A copy thereof is attached as Exhibit L-174.

On or about September 9, 1995, Roger Lasken prepared *Tth* polymerase for shipment. This activity was recorded on page 98 of notebook 3903. A copy thereof is attached as Exhibit L-175.

#### IV. Conclusion

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom.

Further, declarant sayeth not.

Date: 11/29/01

Name: Deb Kumar Chatterjee

Signature: Deb K Chatterjee

## **CURRICULUM VITAE**

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### **Personal**

Date of Birth: December 4, 1953

Citizenship: USA

### **Education:**

Ph.D. (Biochemistry) 1981, University of Calcutta

### **Duties and Responsibilities**

The duties and responsibilities include (a) Generation of ideas for the development of novel products and technologies for Functional Genomics and Proteomics research, (b) Evaluation (feasibility studies) of new ideas generated in-house or technologies to be licensed from outside, (c) Cloning and expression of proteins by protein engineering and using proper expression host(s), (d) Developing purification protocols that must be adaptable to and scalable for manufacturing, (e) setting specifications, (f) Production of proteins at manufacturing scale in conjunction with manufacturing personnel before final transfer to Manufacturing and (g) Stay in contact with Manufacturing for troubleshooting and guidance.

### **Managerial Leadership**

I have fifteen years of experience in directing and supervising various groups of scientists. Currently, directing and supervising a group scientist to develop programs leading to new products and technologies with the objectives described above. Maintain high standards in the planning, design and execution of programs. Responsible for budgets and carrier development for the group. Responsible for creating an environment for scientific professionals that fosters scientific excellence. Acting as an advisor to top management.

### **Technical Leadership**

Expertise in Molecular Biology, Protein Engineering, Gene Expression in prokaryotic and eukaryotic systems, Protein Purification, Microbial Genetics and Recombinant DNA Techniques, and DNA Sequencing etc, Proven record of excellent research accomplishments. Ability to work independently, to provide leadership and to work in a collaborative setting. Ability to accept new challenges and working hard.

### **Professional Experience**

## **Professional Experience**

2000-	Senior Research Fellow (Director level), Invitrogen/Life Technologies.
1992 - 2000	Research Fellow, Life Technologies.
1991 - 1992	Principal Scientist, Life Technologies.
1988 - 1991	Group Leader, Life Technologies.
1986 - 1988	Senior Scientist, Life Technologies.
1985 - 1986	Visiting Scientist, EPA, Gulf Breeze, Florida
1979 - 1985	Post-doctoral fellow in the laboratory of Prof. A.M. Chakrabarty, University of Illinois, Chicago.

## **Awards/Honors**

Two consecutive President Awards for excellence in leadership (1998, 1999)  
Two Dexter Awards for outstanding Technical Innovations (1992, 1996)  
Research Fellowship Award, Government of India  
National Merit Scholarship Award, Government of India

## **Professional duties**

Regular reviewer of Journal articles and Research grants.  
Invited speaker - Universities, Research Conferences.

## **Patents**

21 Patents  
6 Patents submitted

## **List of Publications:**

1. Ghosh, J.J., Mitra, G., Poddar, M.K. and Chatterjee, D.K. (1977). "Effect of  $\Delta^9$ -tetrahydrocannabinol administration of hepatic functions." Biochem. Pharmacol. **26**, 1797-1801.
2. Guha, M., Chatterjee, D.K., Hati, R. and Datta, A.G. (1978). "Existence of an inhibitor of iodination reaction in sheep sub-maxillary extract." J. Ind. Chem. Soc. **LV**, 103-104.
3. Chatterjee, D.K., Banerjee, R.K. and Datta, A.G. (1980). "Studies on peroxidase catalysed formation of thyroid hormones on a protein isolated from sub-maxillary gland." Biochem. Biophys. Acta. **612**, 29-39.
4. Chatterjee, D.K., Kellogg, S.T., Hamada, S. and Chakrabarty, A.M. (1981). "Plasmid specifying total degradation of 3-chlorobenzoate by a modified ortho pathway." J. Bacteriol. **146**, 639-646.

*Pathogenicity and Ecology of Bacterial Plasmids*, S. B. Levy, Royston C. Clowes and E. Koenig, Eds., Plenum Publishing Corp., New York, 519-528.

6. Kellogg, S.T., Chatterjee, D.K. and Chakrabarty, A.M. (1981). "Plasmid assisted molecular breeding - New technique for enhanced biodegradation of persistent toxic chemicals." Science, 214, 1133-1135.
7. Chatterjee, D.K. and Chakrabarty, A.M. (1981). "Plasmids in the biodegradation of PCB's and chlorobenzoates." IN: *Plasmids in the Biodegradation of Xenobiotics and Recalcitrant Compounds*, T. Leisinger, A.M. Cook, J. Nuesch and R. Hutter, Eds., Academic Press, London, 213-219.
8. Chatterjee, D.K., Furukawa, K. and Chakrabarty, A.M. (1981). "Interactions of plasmids in the total degradation of synthetic environmental pollutants." Indian Biologist, 13, 1-11.
9. Chatterjee, D.K., Kellogg, S.T., Furukawa, K., Kilbane, J.J. and Chakrabarty, A.M. (1981). "Genetic Approaches to the Problems of Toxic Chemical Pollution." IN: *Recombinant DNA*, A.G. Walton, Ed., Elsevier Scientific Publishing Company, Amsterdam., 199-212.
10. Karns, J.S., Kilbane, J.J., Chatterjee, D.K. and Chakrabarty, A.M. (1981). "Laboratory Breeding of a Bacterium for Enhanced Degradation of 2,4,5-T." IN: *Genetic Engineering for Biotechnology*, J. Cromoco, Ed., Promocet, Sao Paulo, Brazil, 37-40.
11. Kilbane, J.J., Chatterjee, D.K., Karns, J.S., Kellogg, S.T. and Chakrabarty, A.M. (1982). "Biodegradation of 2,4,5-T by a pure culture of *Pseudomonas cepacia*." Appl. Environ. Microbiol., 44, 72-78.
12. Chatterjee, D.K., Kilbane, J.J. and Chakrabarty, A.M. (1982). "Biodegradation of 2,4,5-T in soil by a pure culture of *Pseudomonas cepacia*." Appl. Environ. Microbiol., 44, 514-516.
13. Chatterjee, D.K. and Chakrabarty, A.M. (1982). Genetic rearrangements in plasmids specifying total degradation of chlorinated benzoic acids. Mol. Gen. Genet., 188, 279-285.
14. Chatterjee, D.K. and Chakrabarty, A.M. (1983). "Genetic homology between independently isolated chlorobenzoate degradative plasmids." J. Bacteriol., 153, 532-534.
15. Chakrabarty, A.M., Karns, J.S., Kilbane, J.J. and Chatterjee, D.K. (1983). "Selective Evolution of Genes for Enhanced Degradation of Persistent Toxic Chemicals." IN: *Genetic Manipulation - Impact on Man and Society*. W. Arber, W.J. Peacock, K. Illmensee, and P. Starlinger, Eds., ICSV Press, Miami, Florida.
16. Kilbane, J.J., Chatterjee, D.K. and Chakrabarty, A.M. (1983). "Detoxification of 2,4,5-T from contaminated soil by *Pseudomonas cepacia*." Appl. Environ. Microbiol., 45, 1697-1700.



17. Chatterjee, D.K. and Chakrabarty, A.M. (1984). "Restriction mapping of chlorobenzoate degradative plasmid and molecular cloning of degradative genes." Gene, 27, 173-181.
18. Karns, J.S., Kilbane, J.J., Chatterjee, D.K. and Chakrabarty, A.M. (1984). "Microbial Biodegradation of 2,4,5-Trichlorophenoxyacetic acid and chlorophenols." IN: *Genetic Control of Environmental Pollutants*, G.S. Omenn and A. Hollaender, Eds., Plenum Press, New York, New York, 3-21.
19. Ghosal, D., You, I.S., Chatterjee, D.K. and Chakrabarty, A.M. (1985). "Genes specifying degradation of 3-chlorobenzoic acid in plasmids pAC27 and pJP4." Proc. Natl. Acad. Sci. 82, 1638-1642.
20. Ghosal, D., You, I.S., Chatterjee, D.K. and Chakrabarty, A.M. (1985). "Microbial degradation of halogenated compounds." Science, 228, 135-142.
21. Ghosal, D., You, I.S., Chatterjee, D.K. and Chakrabarty, A.M. (1985). "Plasmids in the degradation of chlorinated aromatic compounds." IN: *Plasmids in Bacteria*, D. Helinski, S.N. Cohen, D. Clewell, D. Jackson and A. Hollaender, Eds., Plenum Press, New York, New York, 667-686.
22. Tomasek, P., Frantz, B., Chatterjee, D.K. and Chakrabarty, A.M. (1986). "Genetic and Molecular Basis of the Microbial Degradation of Herbicides and Pesticides." IN: *Biotechnology for Solving Agricultural Problems*, P.C. Augustine, H.D. Danforth, M.R. Bakst, Eds., Nijhoff, Dordrecht, 355-368.
23. Frantz, B., Ngai, K.L., Chatterjee, D.K., Ornston, L.N. and Chakrabarty, A.M. (1987). "Nucleotide Sequence and Expression of *clcD*. A plasmid-borne dienelactone hydrolase gene from *Pseudomonas*." sp. B13. J. Bacteriol., 169, 704-709.
24. Chatterjee, D.K. and Bourquin, A.W. (1987). "Metabolism of aromatic compounds by *Caulobacter crescentus*." J. Bacteriol., 169, 1993-1996.
25. Chatterjee, D.K. and Chatterjee, P. (1987). "Expression of degradative genes of *Pseudomonas* in *Caulobacter crescentus*." J. Bacteriol., 160, 2962-2966.
26. Deretic, V., Chandrasekharappa, J.F., Gill, J.F., Chatterjee, D.K. and Chakrabarty, A.M. (1987). "A set of cassettes and improved vectors for genetic and biochemical characterization of *Pseudomonas* genes." Gene, 57, 61-72.
27. D'Alessio, J.M., Hammond, A.W. and Chatterjee, D.K. (1988). "TG Enrichments: A method for subcloning structural genes into expression vectors." Gene, 71, 49-56.
28. Hammond, A.W., Gerard, D.G., Campbell, J.H. and Chatterjee, D.K. (1989). "Characterization of a restriction enzyme from *Neisseria gonorrhoea* which recognizes 5'G<sup>+</sup>CCGGC3', An isochizomer of *NaeI*." Nucleic Acids Research, 17, 3320.

29. Hammond, A.W., Gerard, G. and Chatterjee, D.K. (1989). "Characterization of *NgoAIII*, An isoschizomer of *SstII* from *Neisseria gonorrhoea*." Nucleic Acids Research, 17, 6750.
30. Chatterjee, D.K., Fujimura, R., Campbell, J. and Gerard, G. (1991). "Cloning and overexpression of gene encoding bacteriophage T5 DNA polymerase." Gene, 97, 13-19.
31. Hammond, A.W., Gerard, G.F. and Chatterjee, D.K. (1991). "Cloning the *KpnI* restriction-modification system in *E. coli*." Gene, 97, 97-102.
32. Chatterjee, D.K., Hammond, A.W., Blakesley, R.W., Adams, S., and Gerard, G.F. (1991). "Genetic organization of *KpnI* restriction-modification system. Nucleic Acids Research 19, 6505-6509
33. Smith, M., Longo, M., Gerard, G. and Chatterjee, D.K. (1992). "Cloning and expression of *PvuI* restriction-modification systems in *E. coli*." Nucleic Acid Research 20, 5743-5747.
34. Shandilya, H., and Chatterjee, D.K. (1995). An engineered thermosensitive Alkaline Phosphatase for dephosphorylating DNA. Focus, 17(3) 93-95.
35. Flynn, E., Oberfelder, R. W. and Chatterjee, D. K. (1997). Protein Analysis with the BenchMark Protein Ladders. Focus, 19(2) 33-35.
36. Yang, S; Astatke, M; Potter, J and Chatterjee D.K (2001). A novel mutant DNA polymerase for SNP analysis and detection. Submitted - J. Biol..Chem.
37. Shandilya, H., Yang, S. and Chatterjee, D.K. (2001). Mutants of Ribonuclease Inhibitor-- Structure and Function. Submitted -Nucleic Acids Research.
38. Gerard, G; Potter, J; Smith, M; Rosenthal, K; Dhariwal, G; Lee,J. and Chatterjee, D.K (2001). Recombinant Avian Sarcoma Leukosis Virus Reverse Transcriptase Lacking RNaseH Activity is more Thermal Active that Wild-type Enzyme. Submitted - Biochemistry.
39. Astatke, M and Chatterjee, D.K. (2001). A new therapeutic agent: Potential for all retroviruses? In preparation